Plenary Lectures and Young Colleagues Thesis Presentation

EPIGENETIC AND CHROMATIN MODIFICATION IN COLORECTAL CANCER

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Background: A possible alternative pathway of tumorigenesis has been identified for colorectal cancers (CRC). This pathway is associated with serrated precursor lesions, variable levels of microsatellite instability (MSI), driven partly by epigenetic inactivation linked to methylation of CpG island in human colon cancer and often coupled to associated changes in histone state (i.e acetylation, histonedeactylase (HDAC)), chromatin structure and gene silencing. We postulate that epigenetic changes are associated with genetic alteration in colon cancer. Therefore, we studied sporadic CRCs in African Americans (AA) (at higher risk for sporadic CRC) for the expression and methylation status of mismatch repair genes (hMLH1, hMSH2), global acetylation of histone 3 (H3K18), H4 (K12), HDAC2, genetic changes including MSI, BRAF (V600E) mutation and Genome-Wide Array Comparative Genomic Hybridization (aCGH). Methods: MSI experiments were conducted on sporadic CRC in AA (N=95; 56F/39M) using the five markers recommended by the Bethesda guidelines. H3, H4, HDAC2, MLH1 and MSH2 gene expression was checked using an immunohistochemical (IHC) of tissue microarray and MLH1 silencing by methylation specific PCR (MSP) technique. Genome-wide method including CGH microarray (105K) used for chromosome aberration analysis and the BRAF V600E mutation was determined by sequencing.
Results: In AA samples 30% of the cancers demonstrated MSI-H and the mean age for carcinomas was 65.7 years. Most (71.5%) of MSI-H CRC were proximal, moderate differentiated, and highly mucinous. Defects in MLH1 gene were found in 68% CRCs, while this rate was 29% for MSH2. HDAC2 nuclear expression was 81.9%, 62.1%, and 53.1%; for H4K12 acetylation were 71.7%, 61%, and 43.6%, in cancer, adenoma, and normal tissue, respectively. BRAF mutation was observed in 9.7% (8/82) of the cancers. Of MSI-H tumors 20% (7/29) had BRAF V600E mutations and this rate was 12.5% (1/8) for non-MSI. Of the tumors with BRAF V600E mutations, (43%) expressed (by IHC) MLH1. CGH array showed 70% of samples had chromosomal instability (CIN). Of interest in aCGH was amplification of HDAC within chromosome 17 region q21.2-q21.32 that has been implicated in the progression of cancer. Conclusion: We

identified epigenetic and genomic changes in sporadic CRCs from AA patients. HADC2, H4, and MLH1 could be important in sporadic tumors where the effect of epigenetic changes may lead to genetic alteration including CIN and MIN in progression of CRC in African Americans. Our integrative analysis with matched MSI, methylation, CIN and IHC profiles identified epigenetic and genetic alteration in colorectal carcinogenesis and these results to propose several candidate genes that will be helpful to elucidate processes involved in colorectal carcinogenesis.

PREDICTION OF SUBCELLULAR LOCALIZATION IN EUKARYOTES AT THE BASIS OF LARGE SCALE GENOME ANNOTATION

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In this work we present an integrated platform for large-scale eukaryotic genome annotation based on the prediction of subcellular localization, GPI-anchor prediction and membrane protein discrimination into inner and outer classes. Large scale proteomic projects have determined a huge number of aminoacidic sequences whose functions are, in the largest part, still unknown. In eukaryotes compartmentalization plays a major role in intracellular biochemical pathways. However the determination of subcellular localization with experimental highthroughput procedures is a difficult task and computational procedures are needed. We developed BaCelLo (1), a predictor for five classes of subcellular localizations (secretory pathway, cytoplasm, nucleus, mitochondrion and chloroplast) that is based on different SVMs organized in a decision tree. The system exploits the information derived from the aminoacidic sequence and from the evolutionary information contained in alignment profiles. It analyzes the whole sequence composition and the compositions of both the N- and C-termini. The training set is curated in order to avoid redundancy. For the first time a balancing procedure is introduced in order to mitigate the effect of biased training sets. Three kingdom-specific predictors are implemented: for animals, plants and fungi, respectively. When distributing the proteins from animals and fungi into four classes, accuracy of BaCelLo reach 74% and 76%, respectively; a score of 67% is obtained when proteins from plants are distributed into five classes. BaCelLo outperforms the other presently available methods for the same task and gives more balanced accuracy and coverage values for each class. BaCelLo is also described in Nature Protocols, in the Bioinformatics section (2) BaCelLo can be accessed at http://www.biocomp.unibo.it/bacello/. BaCelLo is currently under integration in a workflow which will allow GO functional integration, prediction of GPI-anchors and discrimination between inner and outer membrane proteins. The workflow will be tested on large-scale genome annotation. With a suite of machine learning based methods, developed in house (BaCelLo, SpepLip (3) and ENSEMBLE (4)), we presently built eSLDB (eukaryotic Subcellular Localization DataBase) (5) an online database collecting the annotations of subcellular localization of eukaryotic proteomes. So far five proteomes have been processed and stored: *Homo sapiens*, *Mus musculus*, *Caenorhabditis elegans*, *Saccharomyces cerevisiae* and *Arabidopsis thaliana*. For each sequence, the database lists localization obtained adopting three different approaches: 1) experimentally determined (when available); 2) homology based (when possible); 3) predicted. All the data are available at the website and can be searched by sequence, by protein code and/or by protein description. Furthermore a more complex search can be performed combining different search fields and keys. All the data contained in the database can be freely downloaded in flat file format. The Database is available at: http://gpcr.biocomp.unibo.it/esldb/.

TRACE ELEMENTS IN HEALTH AND DISEASE

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The importance of trace elements for achieving biological process has been now well documented for many years and still many points should be elucidated. They involve in enzyme activities and production of specific materials such as heme and proteins. Many diseases may occur in the absence of trace elements including skin disease and retardation of growth in the absence of Zinc, anemia in the absence of iron and etc. Their overloads may produce toxicity (hemochromatosis) and in wilson disease. However their concentrations in the body should be regularly monitored. All will be discussed in this lecture.

BIOCHEMICAL CHARACTERIZATION OF DERMAL AND EPIDERMAL AUTO-ANTIGENS

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Autoimmune blistering skin diseases are characterized by the presence of high titers of autoantibodies directed against dermal and/or epidermal antigens. Characterization of specific autoantigen-autoantibody system is critical for research as well as for diagnostic and therapeutical purposes. An overview of the different biochemical methods of antigen preparation and the specific methodological applications will be presented with emphasis on type IV collagen. Our group has recently shown that the 5 and 6 chains of type IV collagen are the target antigen in a novel autoimmune disease characterized by sub-epidermal blisters and glomerulonephritis. Type IV collagen molecules form a network structure primarily in the basement membranes of various tissues. To date, six genetically distinct type IV collagen polypeptide chains, 1 (IV)- 6(IV), have been described. The 1(IV) and 2(IV) chains are ubiquitous, whereas 3(IV), 4(IV), 5(IV), and 6(IV) chains are present in a restricted tissue distribution.

PHARMACOLOGICAL EFFECT OF CROCIN: A CONSTITUENT OF SAFFRON

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Crocus sativus L. commonly known as saffron is a perennial stemless herb of the Iridaceae family and widely cultivated in

Iran. Commercial saffron comprises the dried red stigma. Compounds considered pharmacologically active and important in saffron are volatile agents (e.g. safranal), bitter principles (e.g. picrocrocin) and dye materials (e.g. crocetin and its glycoside, crocin). In this study pharmacological effects of crocin such as antioxidant, genoprotective, hypolipidemic and anti-atherosclerosis effects cerebral, renal and skeletal muscle anti-ischemia as well as anticancer activities will be discussed.

MOLTEN GLOBULE-LIKE STATE AND AMYLOID STRUCTURAL STATES OF GLYCATED HUMAN SERUM ALBUMIN

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It is known during hyperglycemia condition (or diabetic condition) glucose reacts spontaneously with proteins especially human serum albumin (HSA) in the process known as a glycation. Here the glycation of HSA in the presence of physiological concentrations of glucose (0-630 mg/dl: 0-35 mM) and unphysiological concentration (500 mM) of different reducing carbohydrates (glucose, fructose and ribose) in various incubation times were studied by structural point of view. Different techniques were used to determine structural changes and extent of HSA glycation such as circular dichroism (CD), fluorescence, microviscometer, transmission electron microscopy (TEM) and tensiometer. The number of moles of glucose bound per mole of HSA (r), the number of reacted lysine and arginine residues, the Amadori product formation and amyloid formation during glycation were determined. Results show after 21 days of incubation under a physiological level of glucose, number of moles of glucose bound per mole of HSA (r) indicated negative values. Therefore, after this incubation time glucose dissociates from HSA confirming the presence of intermediates for this stage. Structural information, Stokes radius, and 1-anilinonaphthalene-8-sulfonate (ANS) binding data measured by fluorescence technique indicated the formation of a molten globule-like state of HSA after 21 days incubation time with 630 mg/dl glucose. Congo red and thioflavin T measurements show the presence and increase of amyloid structure induced in the glycated samples with increasing times of incubation. TEM analysis shows different appearances of amyloid structures (intermediate states) in glycated HSA before finally formation of long straight amyloid fibrils. The surface activities of glycated HSA induced changes in the surface area property of the protein resulting in exposure of the hydrophobic clusters to the solution. This behavior is similar to interactions between proteins and surfactants in the direction of partial unfolding of the protein. Thus, some of the advanced glycation end products (AGEs) can act as a detergent inducing amyloid fibril structures and decreasing CAC (critical aggregation concentration).

GENETIC SELECTION OF STEM CELLS

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Stem cells offer substantial opportunities for providing well-defined differentiated cells for drug discovery, toxicology, and regenerative medicine, but the development of efficient techniques for controlling and directing their differentiation, present a substantial challenge. We developed a new promoter based genetic selection of germline stem cells (GSCs). Germline stem cells, which can self-renew and generate gametes, are unique stem cells in that they are solely dedicated to transmit genetic information from generation to generation. Extensive studies on these two stem cell types in different organisms over the past few years have revealed some commonalities in the mechanisms controlling their self-renewal and differentiation. Furthermore, germline or somatic cells in various organisms and sexes also exhibit their own unique ways of regulating stem cell function. Mouse embryonic stem (ES) cells derive from the inner cell mass of the blastocyst and give rise to the three primitive embryonic layers, which later will form all the different tissue types of an adult. Embryonic stem cells are thus defined as totipotent cells. In vitro, these cells can give rise to all the somatic cells. We developed a strategy for the establishment of germline stem cell lines from embryonic stem cells. These cells are able to undergo meiosis, generate haploid male gametes in vitro and are functional, as shown by fertilization after intra-cytoplasmic injection into mouse oocytes. Molecular and cellular mechanisms underlying differentiation of ES to functional gametes should be elucidated in future research. In other approach, we show that bone marrow stem (BMS) cells are able to trans-differentiate into male germ cells. BMS cell-derived germ cells expressed the known molecular markers of primordial germ cells. The ability to derive male germ cells from ES and BMS cells reveals novel aspects of germ cell development and opens the possibilities for use of these cells in reproductive medicine.

GENETICS OF GERM CELLS: LESSONS FOR STEM AND CANCER CELL BIOLOGY

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Stem cell biology has gained tremendous interest in the recent years, driven by the hope of finding cures for several diseases through transplantation and regenerative medicine. Various types of stem cells have been identified from preimplantation embryos, the foetus, placenta and adult. The isolation of human embryonic stem cells has been considered the biggest breakthrough of the 21st century. Some of these stem cell types are charged with ethical controversies although they are versatile and offer tremendous potential for finding cures for incurable diseases. Different problems and perspectives of stem cell research will be discussed. One of the important issues is the source of stem cells. Recently, we developed stem cells from adults, SSC, which show the most characteristics required for regenerative medicine. Our recent finding revealed that SSCs apparently have the capacity to form pluripotent cells suggests the possibility that these cells can used to generate tissue matched pluripotent cells for regenerative medicine and to study genetic diseases. Different cell types such as vascular-, heart-, liver-, pancreatic-, and blood cells could also be obtained from these stem cells.

Understanding how SSC can give rise to pluripotent stem cells and how somatic stem cells differentiate into germ cells could give significant insights into the regulation of developmental pluripotency as well as having important implications for regenerative medicine. In addition, considering the fact that cancer cells could be transformed from early stem and germ cells, possibly due to environmentally induced alterations of the niche, we discuss potential linked between germ cells, cancer and stem cells. Understanding how a germ cells can give rise to a pluripotent stem cell could give significant insights into the regulation of developmental totipotency as well as having important implications for male fertility and the aetiology of cancer.

CELLULAR AND EXTRACELLULAR MATRIX RESPONSES IN VASCULAR REMODELING: POTENTIAL MECHANISMS FOR RESTENOSIS AFTER BALLOON ANGIOPLASTY AND STENTING

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In the today society, coronary artery disease (CAD) is a major cause of morbidity and mortality. The introduction of the electrocardiogram during the first half of 20th century led to major clinical advances in the definition of CAD and allowed the development of treatment modalities such as percutanerous transluminal coronary angioplasty (PTCA) and stenting. In the late 1970's and early 1980's, PTCA was seen as the best possible alternative to coronary artery bypass graft surgery. However, 30% to 60% of patients demonstrate restenosis within 6 months following angioplasty. To overcome this major drawback of PTCA, stent implantation was introduced in 1985. Although stent implantation reduced the incidence of restenosis to 20% to 30% of stented vessels, in-stent stenosis remains a major clinical problem. The predominant focus of our studies has been the cellular and extracellular matrix responses in vascular remodeling, particularly cell proliferation, cell migration, collagen synthesis and accumulation, proteoglycans and proteolytic enzymes. Following PTCA or stent implantation, repair processes are initiated. In the early phase of arterial repair there are inflammatory infiltrates and a dramatic increase in cell proliferation, collagen synthesis and protease activities that peaks at 1 week after injury. Despite the marked reductions in cell proliferation and collagen synthesis during the later phase, intimal thickening continues mainly due to increase in extracellular matrix protein accumulation. Using animal models, several novel compounds such as matrix metalloproteinase inhibitors, proteoglycans and cyclin-dependent kinase inhibitors have been assessed by drug therapy and gene delivery approaches to alter the repair response. These approaches significantly reduced intimal hyperplasia demonstrating a potent beneficial effect of these compounds for the prevention of restenosis. In the recent years stents coated with drug-delivery vehicles have been developed to deliver drugs that were known to interrupt the biological processes that caused restenosis. In the data gathered so far, the drug-eluting stent has been extremely successful in reducing restenosis. Ultimately, these advances offer a great hope for markedly lower rates of restenosis. However, given the prevalence of coronary artery disease, the number of percutaneous coronary interventions will continue to increase. Therefore, expanding our understanding of restenosis and treatment options becomes more pressing especially in the conditions such as multivessel disease and chronic total occlusions that may complicate interventions.

BACTERIAL FLAGELLA GLYCOSYLATION IN AEROMONAS SPECIES

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Most mesophilic Aeromonas species produce two distinct flagella systems. In liquid, aeromonads are motile through a single polar flagellum (Fla), but on a solid surface they express an entirely distinct lateral flagella (Laf) system that is used for swarming. The polar flagellum requires over 40 genes for its biogenesis and assembly whereas lateral flagella need around 35 genes. The flagellins (structural proteins of the filament) of each flagella system migrate at an aberrant size on SDS-PAGE and are thought to be post-translationally modified. In Aeromonas caviae Sch3N the genes required for this modification are located in the flm locus, rmlB, flmA, flmB, neuA, flmD, neuB, lsg and lst. The predicted function of the translated products shared homology to proteins involved in polysaccharide biosynthesis or protein glycosylation. The locus was flanked by two transposon-like elements and had a low G + C (42%) content for Aeromonas (normally 60%) and is believed to have been acquired by horizontal transfer. Non-polar insertion mutants were created in all eight genes and they all demonstrated phenotypes of non-motility, lack of LPS O-antigen and flagella with the exception of the mutants in lsg and lst that only lacked their LPS O-antigen. The results suggest a locus with dual function with the genes lst and lsg being involved in LPS assembly only, whereas flmA, flmB, neuA, flmD and neuB are involved in saccharide biosynthesis that is required for both the LPS and flagella glycosylation. In the Aeromonas hydrophila AH-3, the orthologous genes are required for flagella only. Mass spectrometry of A. caviae polar flagellin tryptic digests has demonstrated that aeromonad flagella are indeed glycosylated with 6 – 10 residues of pseudaminic acid (5,7-diacetatamido-3,5,7,9-tetradeoxy-L-glycero-L-manno-non-ulosonic acid) a nine-carbon sugar that is related to sialic acid (Neu5Ac). Mass spectrometry has also shown that this unusual sugar is also missing from the LPS of the A. caviae flm locus mutants. Pseudaminic acid is found on the flagella of Helicobacter pylori and Campylobacter jejuni. Bacterial flagellin proteins interact with toll-like receptor-5 (TLR-5) on host cells and stimulate an inflammatory response. This results in interleukin-8 (IL-8) release, a measure of cell stimulation. Therefore pseudaminic acid on aeromonad flagella may mask these structures from the immune system. Aeromonad flagellins with and without pseudaminic acid additions were engineered and tested on the human cell line Caco-2, which has the TLR-5 receptor. Both unglycosylated flagellins, (FlaA and FlaB), were confirmed to stimulate IL-8 production and up-regulation of TLR-5 expression.

APPLICATIONS OF GENETIC ENGINEERING TO AGRONOMY : WHAT ARE THE ADVANTAGES OF GENETICALLY MODIFIED PLANTS ?

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Genetically modified (GM) plants, or transgenic plants, can be obtained by direct gene transfer, or using as a vector a disarmed Ti (Tumor inducing) plasmid, whose oncogenes have been replaced by the gene(s) of interest. A GM plant therefore contains in its genome one or (or several) additional gene(s) conferring new properties. Up to now, genes introduced into plants mainly confer resistance to herbicides, or to pathogens (such as insects, or viruses), but resistance to abiotic stresses (cold, heat, drought, salt) has also been considered and, in some cases, achieved. More recently GM plants have been obtained which have improved nutritional qualities, such as plants containing higher proportions of unsaturated fatty acids in their oil (to prevent cardio-vascular diseases), or rice containing beta-carotene (to prevent vitamin A deficiency), or plants with a higher content in essential amino acids (lysine, methionine), or iron (to prevent anemia). GM plants have also been developed to produce large quantities (molecular farming) of proteins of therapeutic interest (such as vaccines) without contamination by viruses or prions, or of substances of industrial interest (such as secondary metabolites, starch with various amylose/amylopectin ratios, biodegradable plastics, bio-fuels, etc…) No adverse effect of GM plants on human health has been documented, although millions of people have been eating GM plants for many years. The risk that GM plants, or products derived from these plants, might cause allergies also exists with non-GM plants obtained by conventional breeding, and should be evaluated on a case-by-case basis. Risks for the environment have also been mentioned, particularly as a consequence of gene flow, but this phenomenon can be controled. The appearance of insect strains resistant to the insecticide produced by the GM plant can also be prevented by providing refuges containing untransformed plants, on which insects can feed without being under a selection pressure which might lead to the appearance of a resistance. Further research is certainly necessary, so that a science-based evaluation of benefits vs risks can be made for both GM and non-GM plants, in order to allow the rational discussions needed for public acceptance of the best solutions to the problems which sustainable agriculture is facing today.

THREE PROTEINS, MBNL, MBLL AND MBXL, CO-LOCALIZE *IN VIVO* WITH NUCLEAR FOCI OF EXPANDED-REPEAT TRANSCRIPTS IN DM1 AND DM2 CELLS

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Myotonic dystrophy is a complex neuromuscular disorder associated with DNA expansion mutations in two different genes. In DM1 a CTG repeat in the 3'-untranslated region of DMPK is expanded, whereas in DM2 an intronic CCTG expansion occurs in the gene ZNF9. Transcripts containing expanded repeats form foci in the nuclei of DM1 and DM2 cells. Recent work using antibodies has shown that proteins related to Drosophila muscleblind co-localize with repeat foci in DM1 and DM2 cells. We show that rather than there being a single human muscleblind gene producing multiple proteins through alternative splicing, there are in fact three different muscleblind genes, MBNL, MBLL and MBXL, which map to chromosomes 3, 13 and X, respectively, and which show extensive alternative splicing. Two of the genes, MBNL and MBLL, are expressed in many adult tissues whereas MBXL is expressed predominantly in the placenta. Green fluorescent protein-tagged versions of MBNL, MBLL and MBXL co-localize with nuclear foci in DM1 and DM2 cells, suggesting that all three proteins may play a role in DM pathophysiology.

THE CHAPERONE ACTION OF A-CRYSTALLIN

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α-Crystallin is the principal lens protein, which acts as a molecular chaperone by stabilizing proteins under stress conditions. Macromolecular crowding is ubiquitous and the normal condition in all types of cells. While much *in vitro* work has been published on the interactions of α-crystallin with target proteins in dilute solutions, here, its interaction with a range of destabilized proteins in the presence of dextran (68 kDa) has been examined using visible absorption spectroscopy, tryptophan fluorescence spectroscopy, ANS binding, TEM, HPLC and NMR spectroscopy studies. In the presence of dextran, the rate and extent of aggregation of reduced ovotransferrin, insulin, α-lactalbumin and β_L-crystallin was accelerated. Under these conditions, α-crystallin was less effective in preventing aggregation and precipitation of target proteins. A kinetic competition may exist between aggregation of target proteins and the chaperone action of α-crystallin, supporting the hypothesis that α-crystallin interacts more effectively with slowly aggregating rather than rapidly aggregating target proteins. Amyloid fibril formation by α-lactalbumin, α_s- and ß-casein was verified by a sigmoidal increase in Thioflavin T fluorescence over time. α-Crystallin prevented amyloid formation in α_s- and ß-casein. In the presence of dextran, the rate of amyloid formation by α-lactalbumin, α_s- and ß-casein was enhanced. Under these conditions, α-crystallin was less effective in preventing amyloid formation of ß-casein and this was supported by TEM, CD, NMR spectroscopy and HPLC studies.

BIOCHEMICAL AND DIETARY FEATURES OF PATIENTS AT CORONARY RISK: A FOCUS ON TRACE ELEMENT AND ANTIOXIDANT STATUS AND HEAT SHOCK PROTEIN ANTIBODY TITRES

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Coronary heart disease (CHD) is a common multi-factorial disease. It is influenced by dietary factors, and inflammatory markers appear to predict outcome. The dietary intake of patients at coronary risk and controls was assessed by food frequency questionnaire. Dietary macro- and micronutrient intake was related to plasma antibody titres to heat shock proteins (Hsp). Dyslipidaemic patients (n=238) were recruited from the Lipid Clinics of a local NHS hospital, Guildford, UK. Demographic features, including the prevalence of CHD risk factors such as hypertension and obesity, were typical of Lipid Clinic population. Controls (n=189) were recruited from hospital and university employees. The dyslipidaemic patients were found to have a significantly higher dietary intake of protein ($p<0.05$), starch ($p<0.05$), fiber ($p<0.05$), total fat ($p<0.05$), selenium ($p<0.05$), zinc ($p<0.05$), and higher dietary zinc/copper ratio, compared to controls. These patients also had significantly higher serum copper ($p<0.001$), copper/ caeruloplasmin ratio ($p<0.01$), and selenium ($p<0.05$), concentrations and lower GPx ($p<0.001$), and zinc/copper ratio ($p<0.05$) than controls. Serum selenium concentrations decreased with accumulating features of the metabolic syndrome within the dyslipidaemic subjects ($p<0.05$). Among dyslipidaemia; obesity and presence of the metabolic syndrome contributed significantly to serum C-reactive protein (CRP) concentrations. CRP concentrations increased with accumulating features of the metabolic syndrome ($p<0.01$). Although antibody titres to Hsp-60, -65, and -70 were higher in the dyslipidaemic patients ($p<0.01$), little of the variation in antibody titres could be explained by classical CHD risk factors. Dietary total fat ($p<0.01$), vitamin E ($p<0.05$) and C ($p<0.01$) were major determinants of titres to Hsp-60, dietary vitamin C ($p<0.01$), and vitamin E ($p<0.05$) were major determinants of titres to Hsp-65 ($p<0.01$), and dietary total fat was a determinant of titres to Hsp-70 ($p<0.05$). Treatment of dyslipidaemic patients with statins reduced titres to Hsp-60 ($p<0.05$), -65 ($p<0.01$), and -70 ($p<0.01$), and was also associated with a reduction in serum zinc ($p<0.05$), copper ($p<0.01$), caeruloplasmin ($p<0.05$), and hs-CRP ($p<0.05$).

DIFFERENTIAL GENE EXPRESSION ANALYSIS IN SQUAMUS CELL CARCINOMA OF ESOPHAGUS

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Squamous cell carcinoma of esophagus (ESCC) is one of the most frequent kind of cancer with higher incidenec in special geographic parts of the world. The molecular mechanism of this cancer is still unknown, although it is strongly related to environmental factors, smoking, alcohol, nutritional habits, etc. Using DDRT-PCR technique we found many of genes to be down-regulated in tumor tissues and a moderately fewer number up-regulated. Among the up-regulated cDNAs, one belongs to aldo-keto reductases gene family and shows a clear switching-on in about 63% of tumor tissues. This enzyme is directly involved in polycyclic aromatic hydrocarbon (PAH) compound metabolism, compatible with the cnocept of involvemnet of PAH metabolism in genomic instability and carcinogenesis of esophagus. It is recently known as the most important enzyme induced in oxidative stress and enhances the production of reactive oxygen species and HNE elimination. The other cDNA belongs to MAL gene. Northern analysis showed its down-regulation in about 82% of tumor samples from different stages of ESCC and no clear correlation with methastasis and the patients prognosis. This gene and the third one found in this study, Rab11a, are involved in membrane trafficking and vesicle transport. Rab11a is up-regulated in well differentiated tumors and gets down-regulated in moderately well and poorly differentiated samples. It mediates vesicle recycling of some integral membrane proteins including integrin, a very important component in cell-extra cellular matrix interactions and cell movement. Several other cDNAs with different level of expression according to DDRT films of this study still wait for further analysis.

THE APOPTOTIC AND DIFFERENTIATING EFFECTS OF 3-HYDROGENKWADAPHNIN IN SEVERAL HUMAN LEUKEMIA CELL LINES

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3-hydrogenkwadaphnin (3-HK) is a recently characterized daphnane-type compound isolated from *Dendrostellera Lessertii* with high anti-tumor activity inseveral cancerous cells. Herein, we report on time- and dose-dependent effects of this compound on growth, differentiation, IMPDH inhibition, cell cycle and apoptosis of a panel of human leukemia cell lines (HL-60, K562, NB4, KG1, Jurkat, U937 and Molt4). The drug decreased the growth of leukemia cells in less than 24 h of treatment. However, longer exposure times and/or higher concentrations were required to promote cell apoptosis. Cell cycle analysis revealed the accumulation of cells in their G1 phase as early as 12 h after drug exposure but sub-G1 population was recorded after 24 h. Occurrence of apoptosis was constantly accompanied by morphological and biochemical variations among drug-treated cells. Despite these observations, non-activated normal human PBL were insensitive to the drug action. In addition, treatment of K562 and Molt4 cells with a single dose of the drug for 24 h led to the inhibition of IMPDH activity by almost 50%. Restoration of the depleted GTP concentration by exogenous addition of guanosine (25-50 μM) reversed the drug effects on cell growth, DNA fragmentation and apoptosis. Furthermore, our results were also showed that type II IMPDH as one of the main targets of the drug. Interestingly the effects of the drug in HL-60, NB4 and U937 cells are accompanied with differentiation as well as apoptosis. Indeed, NBT reducing assay, Wright-Giemsa staining, phagocytic activity and expression of cell surface markers (CD11b and CD14)

confirmed that the inhibition of proliferation is associated with differentiation especially toward macrophage-like morphology. Using U937 cells we observed that after drug treatment a number of cells adhere to the culture plates and undergo macrophage differentiation. However, a high portion of cells remain in suspension and showed the characteristics of apoptosis. Apoptosis in these cells was accompanied with p21 fragmentation, and activation of both extracellular (caspase-8 and Fas) and mitochondrial (caspase-9 and Bax) apoptotic pathways. Immunobloting results showed that ERK, JNK and p38 MAPK were activated during treatment of U937 cells with the drug. In contrast, up-regulation of p21, p27 and Bcl-2 inhibited apoptosis and induced plrolonged-G1 arrest in adherent (differentiated cells).These results suggest that 3-HK may be a powerful candidate for treatment of leukemia. These results, in addition of presenting 3-HK as a novel agent for differentiation therapy of leukemia, may be helpful in understanding the unknown pathways in leukemia, thus may pave some novel therapeutical utilities.

RELEASE OF GUT HORMONES IN SICK PEOPLE AND THE AFFECT ON APPETITE AND NUTRITIONAL STATUS

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Recent evidence suggests that a range of peptides released from the gut play a role in appetite regulation. However these gut hormones have not been measured in sick in-patients who are at most risk of malnutrition. I hypothesize that the ghrelin and PYY levels in acutely ill patients will be abnormal and may explain some of the decrease in appetite and poor intake.In ten patients with NOF and sixteen ICU patients fasting concentrations of ghrelin and PYY were measured. Results showed a higher concentration of ghrelin and a lower concentration of PYY during the hospital admission. Concentrations of pre and post test-breakfast ghrelin and PYY were measured in patients with NOF and patients with coronary artery bypass grafting (CABG) to explore a possible mechanism of these hormones on appetite regulation. The test-breakfast suggested a significant and exaggerated postprandial PYY response in patients with NOF compared with control subjects, suggesting a role for PYY in reducing appetite in this group of patients. In patients who underwent CABG the test breakfast suggested a significant and exaggerated postprandial ghrelin decrease on day 6 post operation compared with control subjects, suggesting a possible role for ghrelin in the premature suppression of hunger. The finding of increased PYY over the length of hospital stay and exaggerated postprandial response in sick hospitalised patients is new, and suggests a role in the aetiology of reduced appetite in this patient group. Further studies are required to establish the role of ghrelin and PYY in acute illness.

RELATIONSHIP BETWEEN GLUTATHIONE S-TRANSFERASE- PI MRNA EXPRESSION AND CYCLOOXYGENASE – 2 ACTIVITY IN TISSUE BIOPSIES OBTAINED FROM ESOPHAGITIS, BARRETT'S ESOPHAGUS AND ESOPHAGUS CARCINOMA PATIENTS

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Chronic inflammation in epithelial cells of esophagus is probably the beginning stage of changes leading to malignancy in esophageal epithelial tissue. With introduction of diagnostic endoscopy, biopsy collection and diagnosis of upper gastrointestinal tract has been facilitated. In this study attempts are made to find out the relationship between an inflammatory factor cyclooxygenase-2 (COX-2) and a tumor marker (glutathione S- transferase – Pi; GST-Pi) in human tissues. For this purpose esophagus biopsies were collected from groups of individuals diagnosed with erosive esophagitis(ER), nonerosive reflux disease (NERD), Barrett's esophagus (BE), adenocarcinoma (ADC) and squamous cell carcinoma (SCC). Using these samples, changes in selected molecular markers during development of esophagus malignancy such as P53, P21, nitrotyrosine (NT) expression in esophagus samples were determined. The results showed that in both BE (%60.0) and ADC (66.6%), the incidence of COX-2 was higher as compared to respective normal and ER samples. GST-Pi expression was found to be significantly increased in SCC and ADC. However in case of ER and BE, GST-Pi expression was less affected. Alteration in GST-Pi expression levels was confirmed at mRNA levels using RT-PCR-ELISA showing that GSTpi expression is significantly higher ($P<0.05$) in SCC and ADC samples. Overexpression of P53 was detected in 57.1% and 60.0% in ADC and SCC respectively. P53 showed a much higher expression in BE patients (up to 18.2%) as compared to controls (0.0%) ($P<0.005$). Also P21 showed upregulation in ER (14.3%), BE (20.0%), ADC (52.6%) and SCC (25.0%) biopsies when compared to normal epithelia (0.0%), ($P< 0.05$). NT stained more intensely and in a widespread manner (72.7%) cells in ADC esophageal tissues but about only 36.5% in SCC samples ($P< 0.05$). These data together with endoscopic and pathological observation may suggest that GST-Pi expression although high in esophagus epithelium its expression in induced in ADC and SCC samples. Moreover it appears that COX-2 is responsible for ADC which is likely to develop from BE. In conclusion, P53, P21 and NT staining confirmed previously published data.

ROLES FOR APIS AND THE 20S PROTEASOME IN ADENOVIRUS E1A-DEPENDENT TRANSCRIPTION

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We have determined distinct roles for different proteasome complexes in adenovirus (Ad) E1A-dependent transcription. We show that the 19S ATPase, S8, as a component of 19S ATPase proteins independent of 20S (APIS), binds specifically to the E1A transactivation domain, conserved

region 3 (CR3). Recruitment of APIS to CR3 enhances the ability of E1A to stimulate transcription from viral early gene promoters during Ad infection of human cells. The ability of CR3 to stimulate transcription in yeast is similarly dependent on the functional integrity of yeast APIS components, Sug1 and Sug2. The 20S proteasome is also recruited to CR3 independently of APIS and the 26S proteasome. Chromatin immunoprecipitation reveals that E1A, S8 and the 20S proteasome are recruited to both Ad early region gene promoters and early region gene sequences during Ad infection, suggesting their requirement in both transcriptional initiation and elongation. We also demonstrate that E1A CR3 transactivation and degradation sequences functionally overlap and that proteasome inhibitors repress E1A transcription. Taken together, these data demonstrate distinct roles for APIS and the 20S proteasome in E1A-dependent transactivation.

UBE2Q2, A NOVEL HUMAN GENE, OVER-EXPRESSED IN HEAD AND NECK SQUAMOUS CELL CARCINOMA

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Previous works, performed in our laboratory, screened for genes involved in head and neck squamous cell carcinoma (HNSCC) using differential display (DD) and DNA microarrays. We report here the characterization of one of these novel human genes, UBE2Q2, a putative member of the E2 ubiquitin conjugating enzyme. It encodes a protein of 375 amino acids that contains a RWD domain, a coiled-coil, and an E2 ubiquitin conjugating enzyme domain. UBE2Q2 is up regulated in about 85% of tumor samples. It is expressed in tumor masses and in invasive epithelium, and is located in the cytoplasm of cells. To gain insights into its functions, we identified potential interacting partners by immunoaffinity purification of the flag tagged protein followed by MALDI peptide mass fingerprinting mass spectrometry. Actin and six actin-binding proteins were unambiguously identified as potential interacting partners, suggesting that UBE2Q2's functions may be linked with the cytoskeleton. Preliminary data also showed changes in cell cycle, clonogenicity, cell growth, cell shape and cell-to-cell adhesion. Therefore this novel human gene may represent a new target to develop anti-cancer treatments.

ANALYSIS OF ASSOCIATION BETWEEN BUTYRYLCHOLINESTERASE K VARIANT AND APOLIPOPROTEIN E GENOTYPES IN ALZHEIMER'S DISEASE

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Accumulation of b-amyloid plaques in the brain leads to Alzheimer's disease (AD), a neurodegenrative disorder associated with dementia, unusual behavior, and personality changes. Butyrylcholinesterase-K (BChE-K), a peptidase that participates in the b-amyloid transformation process, and a lipid transporter, apolipoproteinE-e4 (APOE-e4) have been implicated as risk factors associated with AD. In this study we assessed the relationship between BChE-K and APOE-e4 allele with AD in 105 AD cases and 129 age and sex matched controls in Tehran's population, Iran. The frequency of BChE-K allele was found to be significantly different from controls (c^2 =20.6, df=2, $p<0.001$). Computation of the odds ratio as an estimate of relative risk for AD showed that subjects with the BChE-K allele were 2.5 (95%CI=1.64-3.8, $p<0.001$) times more likely to suffer from AD. This risk was increased from 2.37 (95%CI=1.3-4.2, p=0.006) in AD subjects >75 years old to 3.16 (95%CI=1.41-7.1, p=0.001) in subjects 75 years and older. The APOE-e4 allele association risk was found to decrease from 9.5 (95%CI=3.74-24.1, p=0.001) in AD subjects <75 years to 1.36 (95%CI= 0.49-4.1, p=0.58) in those subjects 75 years and older. In addition, a very strong synergic association between BChEK and APOE-e4 (OR=19.1, 95%CI=428-85.45, $p<0.001$) was found in AD subjects. The synergic effect decreased from 36.2 (95%CI=4.4-296, p=0.001) in subjects <75 years old to 6.2 (95%CI=0.9-72.4 p=0.06) in subjects >75 years. These data indicate that the BChE-K and APOE-e4 allele act synergistically to increase the risk of the late-onset AD particularly in age group <75 years in a genetically homogenous population in Tehran, Iran.

STUDY OF PROXISOM PROLIFRATOR ACTIVATED-RECEPTOR- GAMMA, (PPAR-γ) AND TUMOR SUPPRESSOR P53 ROLES IN APOPTOSIS INDUCED BY DOCOSAHEXAENOEIC ACID IN REH AND RAMOS CELLS

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Introduction- Docosahexaenoeic acid is an omega-3 polyunsaturated fatty acid that exerts cytotoxic effect on a variety of cell lines. The molecular target, responsible for mediating this effect of DHA, still remains unknown. In this report, we presented experimental evidences for the role of PPAR-γ in conveying the cytotoxic effect of DHA. Material and method- Apoptosis was assessed by PI analysis and TUNEL method and caspases analysis. mRNA of PPAR-γ and protein levels of PPAR-γ, p53 and caspases were analyzed using RT-PCR and Western blot. Results- we showed that DHA induces apoptosis in Reh and Ramos cells and apoptotic effect of DHA is inhibited by the PPAR-γ antagonist GW9662, indicating that PPAR-γ functions as the mediator of the apoptotic effect of DHA. Furthermore, using western blot method, the present study showed that DHA induces the PPAR-γ protein levels in both Reh and Ramos cells. Moreover, western blot results indicated that caspase3, caspase 9 and PARP protein cleaved by DHA and pretreatment by GW9662 prevented this effect. Interestingly, DHA was found to induce the expression of p53 protein in Reh cells in a PPAR-γ-dependent manner. The up-regulation of p53 protein by DHA kinetically correlated with the activation of caspase 9, caspase 3 and induction of apoptosis, suggesting a role for p53 in DHA-mediated apoptosis in Reh

cells. Conclusion- The present study directly showed apoptotic effect of DHA in PPAR-γ positive cells at least in part exert through this type of nuclear receptor. Since PPAR-γ is ligand dependent nuclear receptor, therefore, study of genes that induce by it was important. We showed that p53 tumor suppressor protein induced by DHA in PPAR-γ dependent manner in p53 wt Reh cells. Taken together, these findings suggest a new signaling pathway, DHA-PPAR-γ-p53, in mediating the apoptotic effect of DHA in hematopoietic cell lines that express functional p53.

PRENATAL DIAGNOSIS OF THALASSEMIA IN IRAN, A NATIONAL SUCCESS STORY

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Thalassemia is the most prevalent single gene disorder in Iran and most part of the world. Now more than 15000 patients live in Iran. National program for prevention of thalassemia started in 1997 and the religious FATWA was given in 1996 to allow prenatal diagnosis (PND). Every couple who wants to get married are tested for thalassemia carriership. If both are carriers or are in doubt of their carrier status are referred to one of several prenatal diagnosis centers throughout the country. Regular visits and inspections are carried out to ensure the best performance. Every PND done is reported to the Genetics Office at CDC. There are more than 10 medical genetics labs in Iran and most of them active in doing PND for thalassemia. Most of these laboratories have been organized as being a network and families are referred to one of these labs via the Health Centers throughout country. In our medical genetics lab. at Kawsar Genomics and Biotechnology Complex we have performed more than 2000 PNDs. We have also analyzed more than 4000 samples referred to us for thalassemia. Only one mistake has been made out of 2000 PNDs which may indicate application of best QA and QC.

1
Clinical Biochemistry

Advanced Methods in Diagnosis and Treatment of Diseases
Clinical Enzymology
Hormonal Disorders
Inborn Error of Metabolism
Trace Elements

Advanced Methods in Diagnosis and Treatment of Diseases

p-1
SELECTION AND USE OF DNA APTAMER FOR DETECTION OF MORPHINE MOLECULE

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Single strand oligonucleotides have different characteristics of which their ability to bind to a particular target makes them a valuable tool for detecting molecules of interest. The study aimed at using morphine as a target to be detected by DNA aptamer. For this purpose a DNA library with two known sequences of 21 and 19 nucleotide at 5' and 3', respectively and the random sequence of 40 nucleotides flanked by the above 2 known sequences were synthesized. The DNA aptamer was amplified with PCR and then mixed with morphine, which was fixed in a solid matrix. After 30 minutes, the reaction mixture was washed with PBS in order to remove the unattached or usably attached aptamer to the morphine molecules. Afterwards, the complex of aptamer-morphine and resin was washed with 7 molar urea in order to remove the strongly attached aptamers from morphine. The DNA aptamers obtained from this step was again amplified by PCR and applied to resin attached to morphine. After 30 minutes it was again washed first with PBS and then with 7 molar urea. This procedure was repeated for 15 times. For the last washing the DNA aptamers were amplified with the fluorescent primers. The PCR product was divided into two equal parts. One part was loaded to the column containing only resin, and the other half loaded to the column containing morphine attached to resin. After washing with PBS and 7 molar urea, we analyzed the attached aptamers with a flowcytomeric method. The difference between the aptamers attached to the column containing no morphine with that of morphine indicate the number of attached aptamers to morphine molecules. After flowcytometry, there was no reading for the column without morphine, while it was 4.5% for the column containing morphine.

O-2
SYNTHESIS OF NANO CRYSTALLINE HYDROXYAPATITE USING A NATIVE BACTERIAL STRAIN

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Hydroxyapatite (HA) is a well-known candidate for many applications in dentistry and medicine such as bone grafts and coatings for medical implants. Studies have shown that nano-structured HA exhibits improved mechanical properties and biocompatibility. Biomineralization is among those of new methods for synthesis of nano structured HA. In recent studies, by using a specific bacterial strain with over producing phosphatase enzyme, nano-crystalline HA has been synthesized. The aim of this study was synthesis of nano-crystalline HA by applying an Iranian bacterial strain. For this purpose, Iranian local strains of Serratia were studied for their enzymatic activity and ability of biofilm developing on inert substrates. After that developed biofilm was exposed to a certain phosphate organic material and calcium salt. During certain time, a white layer produced on the biofilm. Samples were dried and sintered. The characterization of produced layer was performed using scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray diffraction analysis (XRD) and Fourier transform infrared spectroscopy (FTIR). Results showed that one of Iranian strains (PTCC 1187) exhibits high cell-wall phosphates activity. Also it was shown that the synthesized powder was HA with nano-crystalline structure. Furthermore, surface conditions and culture medium criteria for synthesizing maximum HA were determined. The bacterial strain (PTCC 1187) exhibited high ability of producing nano-crystalline HA under optimum conditions. This method may be applied to coat implants of complex shapes.

p-3
THE VALUE OF HIGHLY SENSITIVE CRP COMBINED WITH LDL-C/HDL-C RATIO IN DETECTION OF PATIENTS AT RISK OF CORONARY HEART DISEASE

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Background: It is well known that the elevated high sensitive CRP (hs-CRP) is often associated with endothelial dysfunction and enhanced atherosclerosis. Although recent studies have shown that increased hs-CRP and/or lipoproteins levels are two important risk factors for the development of atherosclerosis, but there are little information about the value of hs-CRP combined with the ratio of LDL-C to HDL-C in the detection of coronary artery disease (CAD). Objectives: The study was designed to evaluate the value of the combined tests in the detection of CAD by measuring the parameters and their correlations in the CAD patients with different levels of stenosis. Materials & Methods: Three hundred male patients (55.7±10.5 years) with CAD referred to Madani Hospital, Tabriz, IRAN was selected. Based on the number of diseased vessels, the patients were divided into three subgroups (66 with one, 87 with two and 110 with three diseased vessels). In all the patients CAD was confirmed by angiography. Control group consisted of 37 apparently healthy age matched males without diseased vessel. The levels of hs-CRP in the serum samples were measured by ELISA and those of lipids and lipoproteins by standard methods using Cobas Mira autoanalyzer. Results: Compared to the control group, significant elevation in serum levels of hs-CRP in patient group was noticed. Significant correlation between the serum levels of hs-CRP, LDL-C, HDL-C and LDL-C/HDL-C ratio was observed (r=0.938). Significant correlations were also noticed between age (r=0.626) weight (r=0.405), systolic blood pressure (r=0.319) and the levels of hs-CRP. Along with an increase in the number of diseased vessels, elevated levels of hs-CRP was found (r=0.927). Conclusion: The direct correlation between serum levels of hs-CRP and LDL-C/HDL-C ratio and number of diseased vessels suggest that detection value of hs-CRP in the CAD increase considerably when evaluated jointly with serum LDL-C/HDL-C ratio. The combined tests can be used as screening test in detection of CAD in general population.

O-4
RELATION BETWEEN HYPERTROPHIC CARDIOMYOPATHY AND MUTATIONS IN THE EXONS OF TNNT2 GENE

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Background: Hypertrophic cardiomyopathy (HCM) is an autosomal dominant disease, which may afflict as many as 1 in 500 subjects (0.2%) in a population. Current data suggest that mutations in the cardiac troponin T (TNNT2) genes are reportedly responsible for up to 40% of familial cases with HCM. Despite lack of any mutational hotspots in this gene, most of the mutations are located in exons 8, 9, 11, 14, 15, 16 of the TNNT2 gene. It is not possible to predict the carriers of mutations in these gene using clinical symptoms, although it is widely accepted that mutations in the TNNT2 gene is frequently linked to sudden cardiac death (SCD). Methods: Amplified target DNA from clinical samples for exons 8, 9, 11, 14, 15, 16 of the TNNT2 gene in HC patients who had a family history of the HC was sequenced. Obtained sequences showed SNP in mentioned exons. Analyses of each DNA sample from individual patients compared to DNA sample analysis in healthy controls through single-strand conformation studies have confirmed the presence of SNP in patients. Results: Mutation in exon 16 (Arg278Cys), exon 14 (Lys247Arg), R278C and 247Arg TNNT2 gene were found in samples that have SNP in exon 14 (Lys247Arg) of TNNT2 gene. Conclusions: Mutations in the exons of TNNT2 gene can be found in patients with or without a family history of HCM and clinical symptoms. However, compared with

healthy populations, a TNNT2 mutation is found more frequently among HCM patients.

p-5
A NEW PRIMER-INDUCED RESTRICTION ANALYSIS FOR RAPID ALPHA-1-ANTITRYPSIN NORMAL VARIANT GENOTYPING

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Alpha-1-antitrypsin (AAT) is the major inhibitor of neutrophil elastase. AAT gene comes in 6 common gene products of M1, M2, M3, M4, S, and Z, 4 of which (M1, M2, M3, and M4) are the most common normal alleles. Two single substitutions in M1 (Arg101 [CGT]-Glu376 [GAA]) are responsible for M3 (Arg101 [CGT]-Asp376 [GAC]) and M4 (His101 [CAT]-Glu376 [GAA]), whereas 2 substitutions in M1 produce M2 (His101 [CAT]-Asp376 [GAC]). PCR-RFLP analysis of the exon II of Arg101/His101 sequence variation using RsaI restriction enzyme can separate M1 and M3 from M2 and M4 alleles. The objective of the study was to directly analyze the exon-V Glu376/Asp376 sequence variation using a designed primer with a single-base substitution (A/T) in its sequence. This substitution induced an artificial site (GT↓AC) for the same restriction enzyme in the PCR product of only M1 and M4 alleles.
M1 and M4 before PCR:5′...ATGATTGAACAAAAT...3′
M2 and M3 before PCR:5′...ATGATTGACCAAAAT...3′
Designed exon-V primer:5′...ATGATTGT-3′
M1 and M4 after PCR:5′...ATGATTGTACAAAAT...3′
M2 and M3 after PCR:5′...ATGATTGTCCAAAAT...3′
M1 and M4 after RsaI Digestion:5′...ATGATTGT and ACAAAAT...3′
M2 and M3 after RsaI Digestion:5′...ATGATTGTCCAAAAT...3′
Two fragments of 163 bps and 22 bps were expected when PCR products of M1 and M4 were digested with RsaI. The findings suggest that the method used is much faster and easier than previous sequencing procedures. Using one enzyme for 2 exons is another advantage of this method, which gives even more cost-effectiveness to it. The method is safer and less labor intensive, as no radioactive compounds are in use.

p-6
SIMULTANEOUS DETECTION OF NEISSERIA MENINGITIDIS AND HAEMOPHILUS INFLUENZAE BY MULTIPLEX POLYMERASE CHAIN REACTION (MPCR) ASSAY

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Meningitis can be caused by either a viral or bacterial infection. Knowing whether meningitis is caused by a virus or bacterium is important because the severity of illness and the treatment differ. Viral meningitis is generally less severe and may resolve without specific treatment, while bacterial meningitis can be quite severe and may result in brain damage, hearing loss, or learning disability. For bacterial meningitis, it is also important to know which type of bacteria is causing the meningitis because antibiotics can prevent some types from spreading and infecting other people. The two major pathogens associated with acute bacterial meningitis are Haemophilus influenzae and Neisseria meningitides. Different methods are used for the detection of H. influenzae and N. meningitides. However, they are less sensitive, take longer, and are more difficult to perform. This study aimed at developing a multiplex polymerase chain reaction (mPCR) assay for the detection of H. influenzae and N. meningitidis. The bacterial strains had been confirmed by biochemical methods. Two primer pairs were designed, being specific to lic-1 gene for H. influenzae and opa gene for N. meningitidis. DNA amplification fragments of 150 bp for H. influenzae and 320 bp for N. meningitides were obtained. Bacterial meningitis such as Streptococcus pneumoniae used as negative control and did not yield a PCR product. This method is rapid, sensitive, specific and can use for detection of H. influenzae and N. meningitides.

p-7
DISTRIBUTION OF STAPHYLOCOCCUS AUREUS ENTEROTOXINS A AND B IN HEALTHY CARRIERS

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The staphylococcal enterotoxins are distinguished for their role in the pathogenesis of human and animal diseases. Various immunoassays based on antigen capture have been developed to identify and assay enterotoxins produced by S. aureus. Among these assays are the reversed passive latex agglutination (RPLA), manual and automated enzyme-linked imunosorbent assays (ELISAs), and immuno-magnetic electrochemiluminescence assays. The issue of cross-reactivity and limited specificity of these assays continue to be a major disadvantage and explain the need for DNA-based toxin identification methods. To determine the distribution of genes that encode enterotoxins A and B, 95 strains of Staphylococcus aureus isolated from healthy carrier were analyzed by Multiplex PCR. Of the total strains studied, only 39 isolates (41%) were diagnosed as sea and seb positive. Twenty four (25.2%) isolates were associated with the sea gene, fifteen (15.8%) isolates were associated with the seb gene and 56 (59%) of these isolates may be possessed other se

genes. The nuc gene, which encodes thermonuclease, was used as a target DNA to identify S. aureus.

p-8
DIAGNOSIS OF HEREDITARY BREAST CANCER BY MULTIPLEX PCR AND MORPHOLOGICAL PARAMETERS

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Background: Hereditary cancers make up 7–10% of all forms of breast cancers. BRCA1/2 mutations determine about 50% of hereditary breast cancer forms and other pathologic BRCA1/2 genotype-associated forms of breast cancer. Analysis of BRCA1/2 mutations is helpful in the determination of developmental potential, early diagnosis and gene therapy for breast cancer. Objective: the objective was to use multiplex PCR with morphological and immunohistochemical parameters in archival breast cancer for detection of BRCA mutations and mtDNA4977 deletion.
Methods: The multiplex PCR was conducted on DNA from 71 archive breast tissue samples and 13 blood samples. Results: Three 5382insC mutations identified from 16 archival familial patients (19%) and 5 mtDNA4977 deletions were detected from 9 blood samples of familial breast cancer patients by multiplex PCR. The mtDNA4977 deletion was highly prevalent in peripheral blood (56%), but it was absent in the breast tissue of cancer cases. Furthermore, hereditary breast cancer tumors exhibited higher mitotic activity, higher polymorphism, lower necrosis, lower tubules, higher ER- and PR-negatives and lower TP53-positives than non-hereditary cancers. Conclusion: The findings demonstrated that DNA extracted by the simple boiling method with microwave yielded higher proportions of successful gene amplifications than DNA extraction Kit. Furthermore, differences in successful gene amplification may be related to size and number of the gene fragment amplified. They also show that testing of mtDNA4977 deletions and 5382insC in combination with morphological and immunohistochemical parameters may be extremely effective and inexpensive tool in testing breast cancer patients aimed to identify individuals with high risk of hereditary breast cancers.

p-9
HIGH INCIDENCE OF 5382INSC MUTATION IN IRANIAN AND UKRAINIAN YOUNG BREAST CANCER PATIENTS

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We identified 136 cases with a personal history of breast cancer diagnosed below the age of 50 from Iran and Ukraine between 2004 and 2005. All cases were tested for the presence of three founder mutations in BRCA1 and BRCA2. The proportion of cases with one of three BRCA mutations (5382insC) was 14% in Iranian young breast cancer patients and 9% in Ukrainian young breast cancer patients. The hereditary proportions were higher than this for women with at least 1 first-degree relative with breast cancer (27%) in Iranian young patients and for women with cancer of ER and PR - negative (19%) in Ukrainian young patients. There was no statistical difference between Ukrainian and Iranian women with breast cancer diagnosed at age <50 years in terms of 5382insC incidence. The findings shows that 5382insC mutation may have originated in the Persia and probably emerged as early as 500 B.C. to Eastern Europe and its testing may be extremely effective and inexpensive tool in testing breast cancer patients aimed to identify individuals with high risk of hereditary breast cancer in Iran and Ukraine.

p-10
EXPRESSION OF EMBRYONIC STEM CELL SPECIFIC GENES IN CO-CULTURES OF EMBRYONIC STEM CELL WITH CORD BLOOD STEM CELL

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Introduction: Mouse embryonic fibroblasts (MEFs) have been used to support the growth of mouse embryonic stem cells (mESc) and human embryonic stem cells (hESCs). Prolonged propagation of mESCs is currently achieved by co-culture with MEFs) to serve as feeder cells. The presence of uncharacterized rodent cells or crude extracts imposes a risk to the clinical applications of hESCs or mESCs. Materials and Methods: Embryonic stem cells were expanded using human USSC, and then expression of CD146, CD29, CD49, VEGFR2, FLK1 were evaluated by flowcytometry and expression of Stat3, BMP4, REX1, Oct4, SOX2, Nanong, Brachyury, Tert, LIF, LIFR Fgf4, were evaluated by RT-PCR and protein expression of Oct4 were evaluated by Immunohistochemestry. Results: Mouse ESC colonies cultured on inactive hUSSCs amplified>600 – fold during 80-day continuous culture (in 30 passage). The expanded mES cells displayed the unique morphology and molecular markers characteristic of undifferentiated mEs cell as observed when they were cultured of MEFs. They expressed oct-4, BMP4, REX1, Nanong, Brachyury, Tert, LIF, LIFR, but not SOX2, Stat3,Fgf4 . Expanded, mES cells on hUSSCs retained unique differentiation potential in culture and a normal diploid karyotype. Conclusion: The findings indicate that co-culture of ESC on cord blood stem cell (USSC) significantly maintain ESCs in the undifferentiated state. Well-studied hUSSCs may provide a clinically and ethically feasible method to expand hEs cells for novel cell therapies.

p-11
SURVEY ON SALMONELLA SPP INFECTION IN SLAUGHTER PIGS IN SASKACHEWAN BY CULTURE AND PCR

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In Canada, salmonellosis ranks as the second most common bacterial foodborne illness in human, and pork and its products remain possible sources of infection. This study aimed at surveying the prevalence of Salmonella infection in Saskatchewan pig slaughter houses using bacteriological culture and PCR. Salmonella spp was isolated from 13.9% (95%CI=9.2%-18.6%) and 5.4% (95%CI=2.3%-8.5%) of the

caecal content and lymph nodes samples, respectively. When PCR was used the prevalence of Salmonella infection from caecal content was significantly higher (32.6%; 95%CI=26.2%-39%). The large differences observed suggested that the accuracy of both techniques should be evaluated on field samples before deciding which one is more advantageous for surveillance purposes. While among all samples analyzed main Salmonella serotypes detected (Derby –23.1%-, Typhimurium var. Copenhagen -15.4%-, and California -7.7%-) were also commonly found in pigs from other areas of Canada, a high prevalence of the serotype Enteritidis (20.5%) was also detected which is commonly associated with infection in humans A larger survey would be advisable to determine the extent of this serotype in the province and its potential implication in human infection. Antimicrobial resistance, although detected to some common antibiotics (ampicilline, chloramphenicol, tetracyclin, and trimethoprim/sulfamethoxazole), appeared to be a lesser problem compared to overall AR results in Canada.

p-12

EXPRESSION, PURIFICATION, CRYSTALLIZATION AND PRELIMINARY X-RAY ANALYSIS OF TWO ARGININE BIOSYNTHETIC ENZYMES FROM MYCOBACTERIUM TUBERCULOSIS

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Despite all the progress in the development of new drugs in the last decades, Tuberculosis (TB) is still a widespread serious chronic disease. TB is caused by the pathogen Mycobacterium tuberculosis (Mtb). More than 1.7 billion individuals worldwide are infected with Mtb. Treatment of TB has become increasingly complicated in the past few decades due to a rise in Mtb strains resistant to existing drugs. The goal of this study was discovering possible targets on which the bacterium depends and then developing inhibitor molecules against these targets. We are studying expressing the proteins from Mtb purifying the proteins and crystallizing them for X-ray crystallographic studies to provide structural basis for the development of new effective therapeutics for Tuberculosis. We report the cloning, expression, purification and crystallization of two enzymes involved in mycobacterial arginine biosynthesis. The RV1652 gene encodes N-acetyl-γ-glutamyl phosphate reductase and RV1656 gene encodes ornithine carbomoyl transferase. The cloning was performed using the Gateway cloning system and expressed in E.coliBL21. The purification processes were done in first step by GST or His-tag then Ion exchange followed by size exclusion chromatography. Initial crystallization conditions for Mtb proteins were found using a hydra plus 1 robot to set up trials with the sitting-drop vapour-diffusion method in 96 well formats. Further structural studies are currently under way.

p-13

CYTOKINES AND NEURO-SPECIFIC PROTEINS AS INDICATORS OF NEURO-INFLAMMATION AND SYSTEMIC INFLAMMATION IN STROKE

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The present work aimed to study the relationship between post-ischemic inflammatory response and the state of blood brain barrier in acute stroke progression in human. The levels of cytokines (interleukin-1beta, interleukin-6, tumor necrosis factor-alpha, monocyte chemoattractant protein-1, and cytokine-induced neutrophil chemoattractant protein), neuro-specific proteins (S100B, neuron-specific enolase) and antibodies to these proteins were determined at different time points in blood samples from patients with acute ischemic stroke complicated and none-complicated with diabetes mellitus type 2. The measurements were performed by ELISA techniques. According to the results obtained a significant increase of the levels of all cytokines and neuron-specific proteins in both groups of the patients was detected on days 1-5 of stroke. Here, the levels of these compounds in diabetes-complicated patients were higher than in those none-complicated with diabetes. It was concluded that neuroprotection of brain blood barrier together with inhibition of cytokines expression might be considered as a new efficient approach in stroke therapy.

p-14

DETERMINATION OF URINARY 8-HYDROXY -2 DEOXYGUANOSINE: A BIOMARKER OF OXIDATIVE DNA DAMAGE UPON KIDNEY TRANSPLANTATION

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Ischaemia-reperfusion injury causes an increased risk of delayed or primary non-function of transplanted grafts during the immediate post–transplant period. Ischaemia-reperfusion has been identified as a key risk factor in predisposing earlier the development of chronic allograft nephropathy and short graft life. Oxidative DNA damage–induced ischaemia-reperfusion injury in the kidney graft immediately after implantation is considered as one major deleterious factor of successful renal transplantation. 8-hydroxy -2´ deoxyguanosine (8-OHdG) is an oxidant of deoxyguanosine, a base for construction of DNA. Oxidative DNA damage accompanied in chronic renal failure is significantly improved after successful renal transplantation, but increased in chronic allograft nephropathy. Twelve women and 13 men who had kidney transplants (35.7±13.67 years) from kidney transplant department were recruited. There was significant difference between the pre and 1 or 2 weeks post transplantation 8-OHdG (26.2±18.6 ng/ml vs. 45.4±21.4 & 57±32 ng/ml, respectively). Plasma urea and creatinine were in normal range. After kidney transplantation the urinary excretion rate of 8-OHdG increased gradually reaching a maximum in two weeks. The increased excretion of 8-OHdG after kidney transplantation may be explained by ischaemia-reperfusion induced oxidative DNA damage of the transplanted kidney.

p-15
INCREASED RATIO OF FREE TO TOTAL LEPTIN IN WOMEN WITH POLYCYSTIC OVARY SYNDRME

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Introduction: Leptin, the product of the ob gene, is involved in the regulation of energy balance and obesity, and circulates in both free and bound forms. The free form of leptin is the most important biologically active form. Polycystic ovary syndrome (PCOS) is associated with insulin resistance and a high incidence of obesity. Objective: the objective was to assess the ratio of free to total leptin in 27 PCOS women (27±5 years) and 27 healthy controls (25±5 years). Methods: Leptin and Insulin were measured using ELISA kits. Free leptin was purified using chromatography Gel filtration and then their fractions were measured using a sensitive ELISA kits. Results: Mean of total leptin and ratio of free to total leptin were significantly different in PCOS subjects and controls (25.89±19ng/ml vs 14.71± 8 and 0.35±0.12 vs 0.26±0.13). Body mass index (BMI) was significantly correlated with leptin (r=0.78) and ratio of free to total leptin (r=-0.51) in PCOS subjects and controls. LH was negatively correlated with total leptin (r=-0.91) and ratio of free to total leptin (r=-0.35). Discussion: The findings indicate that ratio of free to total leptin after subgroup analysis of BMI increased in PCOS patients compared to control ratio of free to total leptin, and circulating total leptin showed similar relation to insulin and BMI and LH in PCOS and control groups.

p-16
MOLECULAR AND IMMUNOLOGICAL EVALUATION OF ANTI-INFLUENZA ACTIVITY OF POLYOXOMETALATE

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Introduction and objectives: Influenza is a common disease among human populations. Attempts have been made to inactivate the virus as a means of disease prevention. Polyoxometalate (POMs) are polymers with antiviral activity in vitro against RNA viruses such as RSV, parainfluenza virus (PFluV) type 2, Dengue fever virus, HIV-1 and SARS coronavirus. POMs are clusters of polyoxonic acid molecules composed of transition metal ions and six oxygen atoms with negative charges. A POM has been developed in Damghan University, Damghan, Iran. To evaluate the anti influenza activity of the POM, FluV A virus was exposed to the POM at various time points and temperatures followed by inoculation of the virus in cell culture. For FluV infection in MDCK cells, POM was added before, after and during infection when the adsorption of virus to cells occurs. Materials and methods: Following treatment of influenza virus with POM at different temperatures and incubation periods, viral titer reduction was assessed by hemagglutination assay (HA). MTT assay was used to determine viability of the cells, antiviral activity of POM on the cell culture, TCID50 of the virus and LD50 of POM. Fluorescein isothiocyanate (FITC) conjugated antibody was used to detect reduction of viral infection by POM. Reverse transcription PCR was performed to evaluate the effect of POM on the viral RNA. Results: The best exposure time and heat condition was 1 h at 37° C .POM could reduce HA titer to zero in all cell culture specimens. 100μM concentration of POM and 10:1 dilution of virus were determined as LD50 and TCID50, respectively. Forty seven percent protection was calculated by SPSS software analysis. Immunofluorescent detection of the cells infected with the virus in presence of different concentrations of POM showed a distinct number of infected cells. RT-PCR results show that RT inhibition activity of POM is similar to the data previously produced in relation to RT HIV-1 inhibitory effect. Discussion: Previous studies have shown that PM-523 inhibits the penetration of Influenza virus to the cell membrane by preventing HA natural conformation during membrane fusion and inhibits the replication of the virus. PM504 also prevents binding of Flu V A to MDCK cells. In this study we show that POM reduces the viral titer after 1 h incubation. Our data suggest that POM has the same effect as PM-523. With regards to POM addition after virus adsorption and HA titer reduction, it is possible that it has some effects on viral replication too .Further investigation will be required to elucidate its mechanism of action.

O-17
ANTI-ANGIOGENESIS PROTEIN FRACTION FROM SHARK CARTILAGE

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Developments of studies on natural therapeutic agents inhibiting the growth of malignant neo-vessels are among the most intensively studied approaches to the treatment of cancer, retinopathies, arthritis and other angiogenesis related diseases. Shark cartilage has proven to have inhibitory effects on endothelial cell angiogenesis, metastasis, adhesion and extracellular matrix proteolysis. In the present study we have optimized a procedure for isolation and partial purification of shark cartilage protein having anti-angiogenesis activity. The protein was isolated by extraction in 4M guanidine hydrochloride buffer followed by Anion and Cation-exchange chromatography and SDS-PAGE electrophoresis. Anti-angiogenesis evaluation was performed using Chick chorioallantois membrane (CAM) and rat aortic ring assay. The results show that the final fraction contains low molecular weight proteins, which are able to block microvessel sprouting in collagen-embedded rat aortic ring assay and capillary sprouting in CAM assay models. It is suggested that these proteins are good candidates for further molecular fine studies in anti-angiogenesis therapies by biotechnological approaches.

p-18
EVALUATION OF RELATIONSHIP OF ALMOND CONSUMPTION AND CORONARY RISK FACTORS

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Risk of coronary heart disease mortality can be decreased by almond consumption. Therefore, it is necessary to study

relationship of almond consumption and some of coronary risk factors. Methods: For this study 30 hyperlipidemic patients were selected. They consumed 60 gram Almond per day for one month. Coronary risk factors such as LDL-cholestrol, HDL-cholesterol, lipoprotein-a, apolipoprotein -A1and apolipoprotein B100 were measured after and before almond consumption. Results: Almond consumption led to the decrease of LDL-cholesterol, lipopoprotein-a, apolipoprotein B100 (LDL-C before=175.08 and LDL-C after=146.04 mg/dl; LP-a before=25.53 and LP-a after=23.42mg/dl; APO-B100 before=119.53 and APO-B 100 after=110.60mg/dl). HDL-cholesterol and apolipoprotein A1 were increased (HDL-C before=33.23 and HDL-C after=43.87 mg/dl, APO-A1 before=133.33 and APO -A1 after=135.07 mg/dl. Conclusion: There is a beneficial relationship between almond consumption and coronary risk factors.

p-19

A STEREOLOGICAL STUDY OF SODIUM TUNGSTATE PROTECTIVE EFFECT ON PANCREATIC BETA CELLS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Type 1 diabetes mellitus results from selective destruction of the beta cells in the pancreatic islets. Hence, search for new agents that protect beta cells from destruction and thereby prevent type I diabetes is necessary. This study investigated protective effect of sodium tungstate against STZ-induced beta-cell damages by means of stereological methods. Sixty rats were divided in to six groups including control (C), tungstate treated control (TC), STZ-induced diabetic (D), STZ-induced diabetic rats treated with sodium tungstate from a week before STZ injection (TDB), food-restricted diabetics (FRD), diabetic rats treated with sodium tungstate one week after STZ administration (TDA). Stereological estimation of pancreas volume, islets volume density, and volume weighted mean islets volume and mass of beta cells, islets, and pancreas and total number of islets were done. Islets volume density, volume weighted mean islets volume and total mass of Beta cells, islets, and pancreas of TDB group was significantly higher than D, FRD and TDA groups ($P<0.001$) and was comparable to controls (C and TC groups). Total number of islets, pancreas wet weight and volume did not show any significant changes between these groups. Results suggested that sodium tungstate preserves pancreatic beta cells from STZ- induced damages and diabetes induction in rats.

p-20

EVALUATION OF DIFFERENT RPE CELL ISOLATION METHODS FROM HUMAN EYE GLOBES

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Visual loss related to retinal dysfunction can often be targeted to the degeneration of the retinal pigment epithelium (RPE), such as in the cases of age-related macular degeneration (ARMD), loss of RPE cells after operations to remove choroidal neovascularization and some cases of retinitis pigmentosa. In these cases, damaged RPE cells culminate to progressive degradation of associated photoreceptors and eventually lead to the observed loss of vision. To inhibit this progressive disease, many approaches have been focused on replacing damaged RPE cells by alternative healthy cells. To achieve this goal, we have examined different RPE isolation methods from human cadaver eye globes, such as physical method and enzymatic method. In regards to physical method two procedures were examined. The first one was the "patch culture method" and the second one was "mechanical separation of RPE cells". For the enzymatic sequestration method dispase I and trypsin-EDTA solutions were compared. Furthermore, DMEM, HBSS and PBS were evaluated as solvents for working enzymes. The data showed that the most effective method for sorting RPE cells, which were more viable, perfect and uncontaminated cells in subsequent cultures, was dispase I enzyme dissolved in DMEM media.

p-21

FORMULATION OF INSULIN IN PEGYLATED NIOSOMES CONTAINING APROTININ: A NEW APPROACH FOR ORAL DELIVERY OF INSULIN

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Investigation on a noninvasive route of insulin administration is a goal for many research groups in the world. The most convenient route for insulin delivery is oral route, but proteolytic enzymes, different pH and mucosal barrier inhibit proper absorption of peptide and protein drugs through this route. In this research PEGylated niosomes containing insulin were evaluated for in vitro protection of this protein against proteolytic enzymes. Niosomes composed of different types of nonionic surfactants and cholesterol was prepared by DRV method. In these niosomes insulin and aprotinin, as an enzyme inhibitor, were entrapped. Thereafter, the size distribution of niosomes and insulin encapsulation efficiency (EE) was evaluated by Malvern size analyzer and RIA, respectively. Also in vitro protection of insulin in the presence of trypsin and pepsin was evaluated in different niosomal formulation and free insulin solution. Results obtained for insulin EE was between 29 to 45 percent and size distribution of vesicular formulation was log-normal. Also incorporation of aprotinin increased the percent of intact encapsulated insulin in the presence of pepsin or trypsin to about 80 percent, whereas free insulin solution was destroyed rapidly. Consequently the high entrapment of insulin in niosomes and good protection of this protein in the enzyme solutions showed the potential of this novel protein delivery system for oral insulin administration.

The future in vivo studies will evaluate the validity of this hypothesis.

p-22
PREPARATION OF A GABAPENTIN POTENTIOMETRIC SENSOR AND ITS APPLICATION TO PHARMACEUTICAL ANALYSIS

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Gabapentin, 1- (aminoethyl – 1 – cyclohexyl) acetic acid, is an antiepileptic drug related to g–butyric acid. Gabapentin crosses the blood brain barrier and is employed for the treatment of partial seizures. Gabapentin has a demonstrated analgesic effect in patients with chronic neuropathic pain states. A novel gabapentin ion–selective electrode is prepared, characterized and used in pharmaceutical analysis. The gabapentin complexes with sodium tetraphenylborate (TPB) and phosphomolybdic acid (MO) were obtained in situ by soaking the PVC-membranes in a 1.0 x 10-3 M gabapentin solution. Among three different solvent mediators tested, dioctyl phthalate (DOP) exhibited a proper behavior including Nernstian slope of 59.8 ± 2 mV decade-1 for gabapentin in the concentration range 1.0 x 10-5– 5.0 x 10-2 M with a limit of detection of 1.0 x 10-5 M. The electrode displays a good selectivity for gabapentin with respect to a number of drugs that may be taken with gabapentin simultaneously. The sensors can be used in a pH range of 1.5–3.2. The membrane sensors were successfully applied to the determination of Gabapentine in its tablets as well as its recovery from blood serum samples. In this paper, we repot a simple potentiometric PVC–membrane sensor for the determination of gabapentin in pharmaceutical preparations. The membrane electrode proposed in this study was made from plasticized–PVC using water – insoluble ion–pair complex, phosphomolybdate (MO)-gabapentin, as an ion–exchanger.

O-23
EFFECT OF IOHEXOL ON ELECTROPHORESIS STUDIES OF TEAR PROTEIN

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Dacryocystography has been widely used in the assessment of the nasolacrimal duct system, particularly in patients with epiphora. This study was performed to determine whether there are differences in tear constituents before and after administration of iohexol. Iohexol is an effective non-ionic and water-soluble contrast agent, used in myelography, arthrography, nephroangiography, arteriography, and other radiographic procedures. Its low systemic toxicity is the combined result of low chemotoxicity and low osmolality. We measured the total protein of tears. The composition of tear protein was assessed by cellulose acetate electrophoresis. For interspecific and intraspecific comparisons of tear protein, sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed on the tear of each sample; this method separated the proteins on the basis of their molecular weights. The data were analyzed by t-test. The concentrations of total protein were similar before and after administration of iohexol. Molecular weights of tear protein bands were similar before and after administration of contrast medium. In this study, iohexol 240 mgI/ml once per day did not cause significant changes in tear proteins.

p-24
EFFECTS OF TRINITROGLYCERIN THERAPY ON SERUM ZINC AND COPPER, AND LIVER ENZYMES IN BALB/C MICE INFECTED WITH LEISHMANIA MAJOR MRHO/IR/75/ER

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The effects of cutaneous leishmaniasis (CL) was investigated on total content of liver enzymes including Alkaline Phosphatase (ALP), Serum Glutamic Oxaloacetic Transaminase (SGOT) and Pyruvic Transaminase (SGPT), essential trace elements (Zn and Cu) and alterations of these contents during successful trinitroglycerine (TNG) therapy in susceptible inbred Balb/c mice .Female mice were divided into three groups, one used as healthy naive, the second group was infected with Leishmania major but untreated as control, the third group comprised of mice infected with L. major and treated with TNG as test group. TNG was inoculated subcutaneously (SC) for fifteen days in the third group. Serum Zn and Cu levels were measured by Flame Atomic Absorption Spectrophotometer (FAAS) and serum liver enzyme (SGOT, SGPT and ALP) concentrations were determined by Auto Analyzer RA1000 .Before TNG therapy; concentration of liver enzymes and copper (Cu) levels were observed to be significantly increased in infected mice compared with healthy naive, whereas zinc (Zn) levels were lower in the infected group than in the controls. These alterations probably indicated to host defense mechanisms against leishmania parasite. TNG therapy caused a change in the levels of liver enzymes, the concentrations of zinc and copper as compared with naive and untreated infected control group.

p-25
SERUM P53 AND BLADDER CANCER: CAN SERUM P53 BE USED AS A TUMOR MARKER?

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Transitional cell carcinoma of the bladder is the second most prevalent malignancy of the genitourinary tract. Recurrence is common with superficial disease, and up to15% of these tumors will progress to muscle invasion. Patients with muscle invasive disease are at high risk for recurrence, progression and metastases. The use of noninvasive and precise methods in the detection and follow up of patients with bladder cancer plays very important role. Mutations in the p53 gene are the common genetic defect in human bladder cancer. The function

of p53 is diverse and complex. Loss of p53 function confers genomic instability, impaired apoptosis and diminished cell cycle restraint. This study included 79 patients with bladder cancer and 79 individuals as controls. Assay of serum p53 protein was conducted using an ELISA kit. There were 41 patients with superficial and 38 patients with invasive carcinoma. The mean levels of serum p53 was30.48 u/ml in superficial patient and55.26 u/ml in patients with invasive carcinoma. This was significantly higher than the mean value (16.91 u/ml) of the controls ($p<0.0001$). Serum p53 rises in patients with bladder cancer and correlates with the grade of the disease. The serum p53 level in bladder cancer patients with grade G1, G2 and G3 was elevated as (19.61±7.60), (36.42±12.90) and (59.78±18.49), respectively. This difference in serum p53 level in different grades is statistically significant. In bladder cancer p53 mutations have been associated with higher tumor grade and advanced stage as well as progression of superficial disease to muscle invasion. P53 has prognostic and therapeutic implications in bladder cancer.

p-26
ORAL SUPPLEMENTS OF VITAMIN E AND BETA-CAROTENE REDUCE LIPID AND PROTEIN PEROXIDATION OF ERYTHROCYTE MEMBRANES IN BETA-THALASSEMIA MAJOR PATIENTS

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Background: Thalassemia is the world's most common hereditary disease. Thalassemia major is the severe form of the disease, presenting with transfusion dependent anemia, generally in the first year of life. Materials and Methods: A prospective double-blind, placebo-controlled study of the effect of vitamin E and beta-carotene on lipid and protein peroxidation in erythrocytes membrane was performed on 120 beta-Thalassemia major patients in four groups. The patients supplemented for 4 weeks as follow: group 1 with beta-carotene (13 mg/day), group 2 with vitamin E (550 mg/day), group 3 with beta-carotene plus vitamin E (13 + 550 mg/day) and group 4 with placebo. We prepared all capsules for 4 groups in same shape and color. Measurement of serum vitamin E and beta-carotene were performed with HPLC. After preparation of ghost cells from blood specimens, malondialdehyde (MDA) and carbonyl compounds were determined as index of lipid and protein peroxidation in erythrocyte membranes before and after vitamin supplementation. Results: The levels of MDA and carbonyl compounds in erythrocyte membranes were significantly decreased after 4 weeks of vitamin supplementation, but in placebo group did not. Group 3 that consumed beta-carotene plus vitamin E, concentration of MDA and carbonyl were decreased more than of group 1 & 2. Conclusions: Our findings provide evidence that an oral treatment with vitamin E and beta-carotene can significantly reduce lipid and protein peroxidation of erythrocyte membranes and could be useful in management of beta-Thalassemia major patients.

p-27
EVALUATION OF ANTI MANNOSIDASE ACTIVITY (A TARGET ENZYME FOR CANCER THERAPY) OF 200 PLANT EXTRACTS

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α-1,2-Mannosidases (class I α-mannosidases) are key enzymes in N-glycan processing in the endoplasmic reticulum (ER) and golgi apparatus , and one of the target enzymes in the development of anti cancer therapies. 1-Deoxymannojirimycin (DMJ), a mannose analogue, specifically inhibits the class I α-mannosidases, resulting in the accumulation of glycoproteins that can induce ER stress leading to the unfolded protein response (UPR). Under UPR, various apoptotic pathways such as CHOP, which is closely associated with cell death, are activated. Also caspase-12, specifically located on the outer moiety of the ER membrane, as a key caspase family member is involved in ER stress mediated apoptosis. Therefore, it is suggested that the inhibition of α-mannosidase I inhibitors play an important role in tumor and HIV treatment. 100 species of plants were collected, and botanically identified. Methanolic and aqueous extracts prepared by maceration method. Enzyme inhibitory effects against α-glucosidase were observed spectrophotometrically at pH 4.5 and 25^{o} C using 0.5mM p-nitrophenyl- α-d-mannopyranoside as a substrate and 1 units/ml enzyme in .02M citrate buffer. Damask Rose and Gallnut plants had more than 50% inhibitory effect on alpha mannosidase. The Kinetic study of these inhibitors showed that the mechanism of inhibition was non- competitive. Km value for this enzyme was 2 mmol/ml, Vmax was 0.05 µmol/min and V¬max in the presence of .25 µg of Gallnut and Damask Rose extracts were0.03 and0.025 µmol/min respectively. Further study should be done in cell lines to show the actual effectiveness of each of these plants.

p-28
PHARMACOKINETIC AND PHARMACOGENETIC ANALYSIS OF WARFARIN IN IRANIAN PATIENTS SENSITIVE TO WARFARIN

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Warfarin is an oral anticoagulant with a greater than 10-fold inter individual variability in dose to attain a therapeutic response. Therefore, patients that use this drug are divided to three groups: normal, resistant and sensitive to warfarin. In this study, we investigated pharmacokinetics of warfarin in sensitive and control patients by HPLC. Then

pharmacogenetics of CYP2C9 enzyme that metabolizes warfarin was analyzed. The analysis performed by HPLC consisted of a column (Perfectsil Target, 5 µm, 125mm×4.0 mm), and an isocratic mobile phase of methanol/acetonitrile/phosphate buffer (pH: 3.5) (7/55/38, v/v), flow rate: 1 ml/min and UV detection at 270 nm. The assay was linear in warfarin concentration ranges of 0.1-10 µg/ml (R2 = 0.9975) and with relative standard deviation (RSD) of <8% for inter-day and <6%for intra-day assays. The mean blood warfarin concentration of 58 patients was 1.4±0.916 µg/ml. The anticoagulant effect of the drug was monitored by international normalized ratio (INR). The correlation of warfarin dosage and concentration with INR was analyzed and that was very poor. Pharmacogenetic analysis of the warfarin metabolic enzyme CYP2C9 confirmed its influence on warfarin maintenance dose. Possession of CYP2C9 variant alleles, which result in decreased enzyme activity, is associated with a significant decrease in the mean warfarin dose.

p-29
RELATIONSHIP BETWEEN HEMOGLOBIN A_2, HEMOGLOBIN F AND RBC INDICES IN BETA THALASSEMIA MINOR

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In most clinical laboratories, evaluation of Hemoglobin A_2 and F are used as a tool for diagnosis of beta thalassemia minor by manual and automated methods. These tests are time-consuming and costly. To see whether there is any relation between these parameters and red blood cells (RBC) indices, this study was performed. Samples from 173 beta thalassemic minor patients were analyzed. Hemoglobin A_2 and F were determined by column chromatography and alkaline denaturation methods, respectively. Both hemoglobins were also measured by electrophoresis on cellulose acetate gel. Blood RBC indices were measured by Sysmex coulter counter. The correlation coefficient between the amounts of hemoglobin A2 obtained by manual method and those fulfilled by automated method was 0.43. The correlation between hemoglobin F obtained by two mentioned methods was 0.95. Based on the results, while there was a reasonable correlation between hemoglobin F obtained by alkaline denaturation method and those obtained by electrophoresis, neither of them showed any correlation with the mean cell volume (MCV) and mean cell hemoglobin (MCH) of patients. The linear relationship between hemoglobin F obtained by manual method with that obtained using electrophoresis, is as follows: Manual method for HbF=0.136+0.917 (Automated method for HbF). No correlation was found between amounts of HbA2 obtained by both methods and neither of them showed any relationship with RBC indices. The findings suggest that either electrophoresis or chemical methods may be used for HbF. Since chromatography for HbA_2 is the reference method, therefore the column chromatography is essential and must be done when performing hemoglobin electrophoresis.

O-30
PRODUCTION AND CHARACTERIZATION OF MONOCLONAL ANTIBODY AGAINST 26 KILODALTON PROTEIN ANTIGEN OF HELICOBACTER PYLORI

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The aim of this study was to establish monoclonal antibody against specific stool antigen or 26 KDa protein antigens of H. pylori. This antibody could be used for diagnosis of this infection in stool of patients. Methods: A 26 KDa protein was purified from whole cell protein of H. pylori by preparative sodium dodecyl sulfate polyacrylamide gel electrophoresis. Three BALB/c mice were immunized by peritoneal injection of the antigen mixed with the same volume of Freund's complete adjuvant and followed with incomplete adjuvant. Highly immunized mice were selected with ELISA and spleen cells were fused with SP2/0 myeloma cells using polyethylene glycol. Fused cells were cultured in hypoxanthine-aminopetrin-thymidine medium.. After screening of clones with ELISA, two stable clones (24H2, 27C7) that produced antibody and reacted with whole cell protein of H. pylori were selected and characterized and blotted with whole cell protein and stool of H. pylori patients. Conclusion: These results indicate that the 24H2 clone would be useful in stool antigen tests for detection of H. pylori in feces.

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COMPARISON OF THE PROTECTIVE EFFECT OF SILYBUM MARIANUM AND EUCALYPTUS WATER EXTRACT ON RAT ALCOHOL-INDUCED LIVER

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Alcohol causes a spectrum of liver injuries that can progress from fatty liver to alcoholic hepatitis to cirrhosis. There is no final treatment for chronic liver diseases. Herbal drugs have become increasingly popular and their use is widespread. The aim of this study is to evaluate the effect of Silybum marianum and eucalyptus water extract on rat alcohol-induced liver. Male albino Wistar rats were treated with ethanol (8 g/kg/day orally) for 60 days, then after 20 days, the animals were treated for 6 consecutive weeks with two herbal extracts and the following parameters were measured: body weight, feed intake, serum proteins, aminotransferase activity, glutathione, alpha-tocopherol and ascorbic acid followed by histopathological observations. Our results showed that these herbal extracts have useful effect on the animals. Also the Silybum marianum extract has better effects than Eucalyptus water extract.

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DEMONSTRATION OF DOSE-DEPENDENT CYTOTOXIC ACTIVITY IN CANCER CELLS BY SPECIFIC HUMAN CHORIONIC GONADOTROPIN MONOCLONAL ANTIBODIES

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Human chorionic gonadotropin (hCG) is synthesized and secreted by placenta and some tumors. Its function is maintenance of embryo in initial embryonic stages and has important roles in angiogenesis, growth, apoptosis, and inhibition and suppression of immune system in tumors. Previous studies indicated that all tumor cells, regardless of their origin and type, express membrane associated hCG in various forms including ß-hCG. On the other hand, Avicin, an anticancer vaccine against CTP-hCG has successfully passed clinical experiments for treatment of cancer patients. In this research hCG monoclonal antibodies (Mab) were used to evaluate their cytotoxic effect on cancer cell lines. These monoclonal antibodies were purified by affinity chromatography using G protein Sepharose. The purity and bioactivity of monoclonal antibodies were confirmed by SDS-PAGE and ELISA. Our results showed that among different antibodies, T5C4 Mab and 7D9 Mab had severe cytotoxic effects on tumor cells. This effect was more prominent on MDA than HeLa cell lines. The findings suggest that the cytotoxic effect of 7D9 Mab on the tumor cell lines may be due to its enzymatic activity. In this regard 7D9 Mab may bind and degrade expressed hCG on membrane of the tumor cell lines and inhibit its functions in cancer cell lines.

p-33
DEVELOPMENT OF IN SITU PCR TECHNIQUE FOR DETECTION OF LATENT HSV-1 DNA IN MICE TRIGEMINAL GANGLIA

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Herpes Simplex Virus-1 (HSV-1) is a neurotropic DNA virus which infects a broad range of cells. This virus causes a number of important human diseases, including gingivostomatitis, pharyngitis, keratitis, and encephalitis. HSV-1 establishes latency in peripheral nerves in which it is maintained for whole life of the host. Periodic reactivation of the latent virus leads to recurrent infections in certain people. In this report, the presence of HSV-1 DNA in trigeminal ganglia of latently infected mice was detected by an indirect in situ PCR technique, based on Digoxigeni11-dUTP detection with anti-DIG-peroxidase and DAB system. BALB/c mice were inoculated through corneal scarification with 5µl drop of medium containing 105.5 TCID50 of wild type HSV-1 in each eye. When latency was established, the mice were killed and trigeminal ganglia were removed. After fixation and paraffin embedding, in situ PCR was performed in 5 µm tissue sections of paraffin embedded ganglia. Based on the results in situ PCR is a very effective method to locate and detect latent HSV-1 within infected neurons compared with the conventional PCR method.

p-34
A MULTIPLEX PCR FOR THE DETECTION OF BRUCELLA SPP. AND SALMONELLA ABORTUS OVIS FROM ABORTED OVINE FETUS

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Brucella spp. and Salmonella abortus ovis are important causes of ovine abortion. Both bacteria can be serologically diagnosed, but many factors may cause false positive and negative results. Direct methods based on bacteriological isolation are usually applied, but they are difficult, time consuming and dangerous. Polymerase chain reaction (PCR) has been successfully described for the detection of Brucella spp. and Salmonella abortus ovis. The aim of the study was to detect such agents aborted ovine fetuses by multiplex PCR. The mPCR was applied to 54 fetal stomach contents, of which 14 were totally negative, 10 were positive for Brucella spp., 24 were positive for Salmonella abortus ovis and 6 were positive for both. Simplicity and the possibility of detection of both bacteria in a single tube reaction support the use of the mPCR in the routine diagnosis.

p-35
THE EFFECTS OF NITRIC OXIDE ON THE HEALING OF BURN WOUNDS IN RATS

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The exact role of nitric oxide (NO) in the wound healing has not yet been well elucidated and the available reports are controversial. The aim was to study the effects of NO on healing of burn wounds in rats. A determined area of the skin of the back region in adult male rats (n=60) after induction of general anesthesia was exposed to boiling water for 8 s to produce wet burn. Then, the animals were grouped randomly in the 6 groups. Control group, which received for 7 days after burn only normal saline intraperitoneally (IP). Experimental groups 1 and 2 received IP, L-Arginine 100 mg/kg, and L-NAME 10 mg/kg, respectively at the first, third and fifth days after burning. Groups 4, 5 and 6 were as above three groups (Control, Experimental 1and 2), but their test period lasted 5 days. They received the drug at the first, third, fifth, seventh,

ninth, eleventh and thirteenth days. The rats of groups 1-3 at the seventh day and the animals of the rest of the groups (4-6) were euthanized on the fifteenth day by ether inhalation. Statistical analyses revealed that the groups receiving L - Arginine had elevated angiogenesis, and this difference was statistically significant. But, the rates of wound healing on different days were not statistically significant. The collagen deposition rate was also enhanced in L-Arginine receiving groups. The inflammation in the L-Arginine receiving groups was less than other groups, but there was no significant difference between study groups in epidermis production rate. The results showed that NO were effective on some of the wound healing indices. Therefore, the induction of NO production in the burns, the extension and progression of the burnto the deeper sites and the occurrence of infection may be prohibited. The main reason of this healing effect may be through facilitating the perfusion to the burn bed.

p-36
EPIDEMIOLOGIC EVALUATION OF VIRULENCE GENES, PAP ,FA, CNF-1, AND HLY IN E. COLI STRAINS ISOLATED FROM CHILDREN WITH URINARY TRACT INFECTION IN IRAN

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There is a complex interaction between different uropathogenic E. coli virulence factors and the host response that determines the outcome of bacterial exposure. To evaluate the prevalence of four important virulence genes, hly, cnf-1, hly and sfa, in E. coli strains isolated from urine samples of children with UTI referred to Motahary hospital, Jahrom, Iran, during Aug 2005-Aug 2006 and their correlation with clinical data, PCR was performed for these four genes. Totally 96 E. coli strains were isolated from urine samples of children with UTI aged 1 month to 14 years (21.8±26.9 months). Cystitis was diagnosed in 49.2% and pyelonephritis in 50.8% of these patients. Prevalence of genes pap, sfa, hly and cnf-1 among the strains was 27.1%, 14.6%, 13.5% and 22.9 %, respectively. Overall 33.3% of samples were positive for at least one of the genes and 6.3% for all four genes. There was significant correlation between age of patients and presence of genes, as pap and sfa were more common in ages over 36 months but hly was more detected in age less than 48 months. Pyelonephritis was more prevalent in cases with positive virulence genes. There was no significant correlation between gender and presence of genes. cnf-1 gene was significantly more common in samples of the patients with abnormal kidney sonography. This study showed that the prevalence of virulence genes hly, cnf-1, pap and sfa in E. coli isolates was similar to the results of other studies. Because of higher prevalence of pyelonephritis in presence of these genes, rapid detection of the genes in urine samples may help in more suspicious and rapid management of pyelonephritis.

p-37
ASSOCIATION OF VIRULENCE GENES HLY, SFA, CNF-1 AND PAP WITH ANTIBIOTIC SENSITIVITIES IN E. COLI STRAINS ISOLATED FROM CHILDREN WITH URINARY TRACT INFECTION

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Recently, some in vitro studies have suggested that decreased pathogenicity of E coli is associated with the acquisition of resistance to some of antibiotics. This study aimed to investigate four virulence factors of pap, cnf-1, sfa and hly in resistant in compare with susceptible strains isolated from UTI in children. Drug sensitivity of 96 E coli isolates was investigated using standard method of disc diffusion. Polymerase chain reaction was used to analyze prevalence of pap, cnf-1, sfa and hly virulence genes. E coli strains showed a high degree of sensitivity to imipenem, amikacin, nitrofurantoin and ciprofloxacin. Approximately 80.2% of the isolates were resistant to ampicilin. Multiple resistances to ampicilin, gentamicin, nalidixic acid and cefixime were seen in 2.1 percent of the isolates, but no case of multiple drug resistance to all drugs was seen. Only 12.5% of the strains were susceptible to all tested antibiotics. Polymerase chain reaction analysis showed that of toxin coding genes under study, cnf-1 (22.91%) was more prevalent than hly (15.62%), of the adhesion coding genes; pap (30.2%) was more prevalent than sfa (18.75%). In all strains the expression of all virulence genes was less prevalent in the most antibiotic resistance group than in susceptible group but not statistically significant except for genotypes of pap+-cnf+, pap+- hly+ and cnf+-hly+ with nalidixic acid. We propose that pap and cnf-1 genes in combination with hly gene constitute an uropathogenic genomic configuration, which is characteristic of the nalidixic-acid susceptible E coli strains causing urinary tract infection.

p-38
RELATIONSHIPS BETWEEN MEASURES OF NITRIC OXIDE PRODUCTION AND ROUTINE MARKERS OF RENAL FUNCTION IN KIDNEY RECIPIENTS

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Assessing circulating surrogate biomarkers of nitric oxide (NO) production such as nitrite, nitrate and L-citrulline in patients receiving renal transplantation is important for our understanding of renal allograft outcome. The aim of this longitudinal study was to examine the relationship between measures of NO production and clinical markers of kidney function such as creatinine (Cr), Blood urea nitrogen (BUN) and C-creative protein (CRP). Recipients of first kidney (age range: 30-60 years, n=27) were recruited. Sampling schedule

commenced every day at day 1 (pre-transplantation) and post transplantation day 1 up to day 14 and thereafter on days 21, 28, 35, 42, 49 and 56, respectively. Plasma nitrite, nitrate and L-citrulline levels were analyzed spectrophotometrically. At pre-transplantation, the mean plasma levels of nitrite, and L-Citrulline were 0.6 and 20 μM, respectively. At day 3 post transplantation, these levels dropped significantly by about 50%. Alteration in L-citrulline concentrations was found to be more pronounced than the corresponding changes in plasma creatinine or BUN concentrations. Significant associations were seen between the plasma measures of NO production and circulating Cr and BUN. These findings suggest measurement of plasma L-citrulline levels may prove a useful diagnostic tool in conjunction with routine markers including Cr and BUN in predicting renal allograft outcome.

p-39
EXPERIMENTAL VISCERAL LEISHMANIASIS IN BALB/C MICE CAN BE INHIBITED BY CYSTEINE PROTEINASE TYPE III

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Visceral leishmaniasis or kala-azar is a chronic systemic disease and could be fatal, if not treated. Vaccination is the only tool to control it. One of the virulence factors of Leishmania infantum is cysteine proteinase type III (CPC), which can be a good candidate for vaccination. In present study, the efficacy of vaccination with cysteine proteinase type III was investigated. CPC gene was isolated from L. infantum genomics by PCR. After sequence confirmation, the CPC fragment was cloned in pQE40 as fused gene with DHFR and then purified using Ni-NTA. Sera reactivities of different stages of cutaneous and visceral Leishmaniasis showed that CPC is highly immunogenic in humans. Prime-boost vaccination was carried out in three groups of Balb/c mice. Test group were primed with pcDNA-cpc and boosted with CPC protein. Control groups received pcDNA and rDHFR or PBS. Mice were challenged with intravenous 2 × L. infantum. Parasite burden were measured in liver and spleen every two weeks after challenge up to fourteen weeks. Humoral immune responses (total IgG, IgG1, IgG2a) were tested before and seven weeks after challenge. Nitric oxide concentrations in peritoneal macrophages were measured seven weeks after the challenge. The results showed that the ratio of IgG2a/IgG1 and nitric oxide concentration is strongly higher in test group than the control group. The parasite load of test group was significantly lower than control. The data indicate that DNA/protein vaccination with CPC can induce an acceptable TH1 response against L. infantum in BALB/c mice.

p-40
FIBRINOLYTIC EFFECT OF SOME FLAVONOIDS

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Disturbance in hearnostatic system cause abnormal clot in vessels and thromboarnbolia. Stereptokinase and tPA are used for emergency treatment of thromboarnbolia. Despite their favorable effects, they have side effects such as general lytic, allergic state, utricaria and serum disease. To find new fibrinolytic compounds with less adverse reaction, there is increasing interest in plant ingredients such as quercetin, naringin, murin, rotin, daidzein, genistein, chaempherol, biochanin A, epigenin. Methods: Fibrinolytic effect was determined based on a fluorometric method. Labeled fibrinogen with FITC was added to plasma. Then Ca^{2+} was added to produce labeled clot. Then streptokinase (100-1000 Iu/ml), flavonoid (10, 50, 100, 200 mg/ml) alone and in the presence of streptokinase, were added. After 15, 30, 45 and 60 minutes flourescence was determined (EX=492, Em=520). A linear relationship between flourescence and concentration of streptokinase (400- 700 Iulml). Quercetin, murin, and narinigin showed significant fibrinolytic effects, but other flavonoids did not have significant effect. In the presence of streptokinase, similar results were obtained. The increase in flourescence of all flavonoids was not time dependent. The data show that quercetin, naringin and murin had synergistic effects with reduced dose of streptokinase. They also suggest performing clinical trials to search for the benefits of this regimen in patients with thrombotic states such as myocardial infarction.

p-41
DETERMINATION OF HELICOBACTER PYLORI IMMUNOGENS BY 2DIMENSIONAL ELECTROPHORESIS AND WESTERN BLOTTING

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Helicobacter pylori is (H. pylori) among common infectious agents that colonize the mucus layer of stomach. This bacterium is an etiological agent of active and chronic gastritis, peptic ulceration and cancer. In order to find immunogens of H. pylori for use in clinical diagnosis, this project was conducted. H. pylori isolated from biopsy of patients suffering from gastritis, peptic ulcer and gastric cancer was extracted by an extraction solution containing lysozyme, urea and CHAPS. Two-dimensional electrophoresis based on isoelectric focusing in rehydrated gel in the first dimensionl and SDS-PAGE in the second dimension was performed. The resolved proteins were transferred to PVDF membrane using tank blotting and reacted against IgG fraction of patient's sera using immunoblotting. The bacterial extract showed several hundred silver-stained spots with molecular weights (MW) from 10 to 100 KDa and isoelectric points (PI) from 3.5-9.5. This pattern contained 6-7 major proteins, some of which belonged to G protein groups and showed several spots. The results of immunoblots revealed that several protein spot with different MW and pI have stained with all three groups of patient's sera and some protein with one or two groups of sera. A protein spot with MW of 30 KDa reacted only with serum of patients with gastric cancer and a protein with MW of 18 KDa only with serum of gastric patients. These proteins are potential candidates for recognition of type of gastric disorder. In addition, the results indicated that protein profile of H. pylori isolated from gastric cancer and

peptic ulcer were more similar than the protein profile of H. pylori isolated from gastritis patients.

p-42
HYBRIDIZATION OF MULTIPLEX PrASE PRODUCTS TO OLIGONUCLEOTIDE SPOTTED MICROARRAYS

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The efficiency of the common Amplification Resistant Mutation Screening-PCR (ARMS-PCR) protocol is often hampered by misextensions of the 3' termini of unmatched primers. Furthermore, the analysis of multiple variant sites can be tedious. We have used a multiplexed PrASE protocol in a microarray analysis system to overcome both problems. PrASE is a modified PCR reaction in which matched and mismatched primers, and the two enzymes DNA polymerase and a protease are all present. The relative kinetics of the polymerase and protease activities are such that extension of perfectly matched primers occurs before the polymerase is degraded. However, because extension of mismatched primers is slower, degradation of the polymerase occurs before extension. Having thus assured specificity, a nested PrASE reaction was developed which allowed simultaneous analysis of several variant positions. The PrASE reaction that was developed included multiplex outer and mutiplex inner PCR reactions. The reactions were designed so that no optimization of reaction conditions was necessary. Products of the inner PCRs were used as templates in the allele specific extension reactions. Fluorescent-labeled products of the PrASE reactions were hybridized to multi-sample microarray slides spotted with universal oligonucleotide probes. The efficacy of this protocol was demonstrated for seven SNP positions within the CFTR gene, which together define intragenic haplotypes of the gene.

p-43
PURIFICATION OF CRP AND ITS ANTIBODY FOR EVALUATING THE CRP LEVEL IN BLOOD AND FOLLICULAR FLUID IN PATIENTS WITH CONTROLLED OVARIAN HYPERSTIMULATION FOR IN VITRO FERTILIZATION/ICSI

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C-reactive protein (CRP) is an acute phase protein synthesized by hepatocytes with the elevation of cytokines Controlled ovarian hyperstimulation (COH) is a key factor in a successful IVF. One of the critical points in COH is ovarian hyperstimulation syndrome (OHSS), which can be observed by elevation in systemic inflammatory cytokines. A competitive ELISA method is developed for quantitative measurement of CRP. The aim of the present study was to determine serum and follicular fluid CRP levels in patients undergoing controlled COH for IVF-embryo transfer cycle, and their possible correlation to COH variables and outcome of IVF. This protein extracted from ascitic fluid by a novel method in this laboratory using calcium phosphate and purified on G 100 gel filtration column. Rrabbits were immunized by the purified CRP and the antibody was purified by salt precipitation and ion exchange chromatography. The subjects were 76 consecutive patients undergoing routine IVF by long GnRH agonist protocol. Blood was drawn four times during the COH cycle; the day on which adequate suppression was obtained (Day-S); the day of HCG administration (Day-HCG); the day of oocyte pick-up (Day-OPU), and the day of Embryo transfer. Levels of follicular fluid CRP were determined. Serum and follicular fluid CRP were measured with a competitive ELISA method. Serum levels of CRP were significantly higher on Day-OPU and Day-HCG than on Day-S, and significantly higher on Day-Embryo transfer than on Day-OPU. Successful outcome is associated with a relative small increment in CRP on the day of embryo transfer than Day-OPU. No difference was observed between follicular and serum CRP levels on Day-OPU. The ELISA results indicated that the serum CRP level increased significantly at COH stage and it seems that the patients reached systemic inflammation at COH stage. The quantitative measurement of CRP can be a diagnostic tool in women treated by IVF. The concentration of CRP in blood increases significantly during the first week following oocyte retrieval.

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BIOCHEMICAL AND MOLECULAR ANALYSIS OF ANTIVIRAL EFFECT OFNANOSILVER

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Introduction and Objectives: Due to nearly annual epidemics, mortalities and morbidities caused by influenza virus and numerous genetic shifts and drifts and in the absence of effective vaccines and drugs, world health authorities have introduced antiseptics to prevent the disease. Nanosilver has been used as a general antiseptic and disinfectant since ancient times and nowadays it has more strong effects in nano-size. So in this study, we are investigating the antiviral effect of nanosilver. Silver does not induce resistance in microorganisms. Methods: MTT colorimetric assay has been used to determine TCID50 of the virus and LD50 of Nanosilver. The presence of the virus genome in the vicinity of Nanosilver in different periods and temperatures was

assessed by RT-PCR. The amount of protein was determined by Lowry method of protein assay. Images of fluorescent microscope using anti-H1N1-FITC antibody in Direct Immunofluorescent Assay (DFA) were prepared. Results: Nanosilver had almost identical preventive effects on the virus in all situations. This effect was confirmed using hemagglutination assay in all samples. Determining the viral RNA and proteins confirmed these results. The immunofluorescent images were the indication of Nanosilver effect on the virus. Discussion: After determining TCID50 of virus and LD50 of Nanosilver and investigating all situations by different biochemical and molecular methods, it was found that Nanosilver can have preventive effect on virus membrane glycoproteins, so it can suppress the entrance phase which is essential to propagation of the virus.

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PROTECTIVE EFFECTS OF WHEY PROTEIN ON THE B_1 AFLATOXIN TOXICITY IN THE HEPATIC ENZYMES AND PLASMA CALCIUM AND MAGNESIUM LEVEL IN MALE RATS

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The Aflatoxin toxicity has negative effects on the value of hepatic enzymes, plasma calcium and magnesium. In this study, the protective effect of whey protein on the B_1 Aflatoxin toxicity was investigated by determining its effect on the value of hepatic enzymes; blood Ca and Mg. Male rats were randomly divided into 3 groups of 10 each. The first group (a; control) was given a particular combination of pelleted food. The second group (b) was given the same food compound as the first group plus 3 mg/kg B_1 aflatoxin .The third group (c) were given the same food as first and second group and they were also treated by 3 mg/kg B_1 aflatoxin and 20 mg/kg whey protein for 8 weeks. Then the blood samples were tested, and the values of hepatic enzymes and minerals were measured. The results show that in group c, the value of ALP, AST, ALT and MDA were 135 UL-1, 207 UmL-1, 53 and 2.7ngmL-1 respectively, that were significantly less than group b (183.8 UL-1,240 UmL-1 and 3.2 ngmL-1). Glutathione peroxidase and blood Glutathione values in the third group, (500 Ug-1Hb and 1.1 µmolg-1Hb) were considerably more than the second group (622 Ug-1Hb and 0.823 µmolg-1Hb). Blood Ca and Mg values were 7.8 mgdL -1 and 1.9 mgdL -1 for group c while they were 7.75 mgdL -1and 1.78 mgdL -1 for group b respectively. The results showed the positive and protective influence of whey protein on blood and hepatic systems against B_1 Aflatoxin toxicity.

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RATIO OF FREE TO TOTAL PROSTATE SPECIFIC ANTIGEN AND TOTAL PROSTATE SPECIFIC ANTIGEN LEVEL TO PROTEIN CONCENTRATION IN SALIVA AND THE COMPARISON WITH SERUM LEVELS IN HEALTHY MEN

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Objective: we investigated free and total prostate specific Antigen (PSA) levels in saliva and compared them with the serum levels in normal men. Methods: Serum and saliva concentration of protein, free PSA (fPSA) and total PSA (tPSA) were measured in 30 healthy men with an age range of (42-73 years, 56.6±9.1). Results: For the total 30 men there were significant positive correlation between serum fPSA/ tPSA and salivary fPSA/ tPSA (r=0.261) and significant positive correlation between serum tPSA/ protein and salivary tPSA/ protein (r=0.3). Although there were positive correlation between salivary tPSA and salivary fPSA (r=0.25) and positive correlation between serum tPSA and serum fPSA (r=0.59). There were significant negative correlation between age and body mass index (BMI) (r=-0.42) and significant negative correlation between serum tPSA/ protein and BMI (r=-0.38) and positive correlation between serum fPSA/ tPSA and BMI (r=0.45). Conclusions: There was significant correlation between serum tPSA/ protein and salivary tPSA/ protein in normal men. There was also a significant correlation between fPSA/tPSA in serum and saliva. The findings might be taken as evidence that it might be possible to use saliva sample instead of serum sample for tPSA determination in normal men.

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COPARISON OF THE CYTOTOXIC AND ANTI INFLAMMATORY EFFECT S OF GLYCYRRHIZA GLABRA AND MATRICARIA AUREA WITH STEROIDAL AND NON-STEROIDAL COMPOUNDS ON MATRIX METALOPROTEINASE ACTIVITY USING FIBROSARCOMA CELL LINES

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The function of matrix metalloproteinase (MMP) in physiology of various inflammatory diseases such as rheumatoid arthritis and malignant tumors has been established. The aim of the present study was to compare the MMP and cytotoxicity effectiveness of Glycyrrhiza glabra and Matricaria aurea with commonly used steroidal and nosteroidal anti-inflammatory agents. Zymographic and cellular cytotoxicity methods were employed, using fibrobsarcoma cell line culture (Whie 164). Glycyrrhiza glabra, diclophenac, dexametheasone and piroxicame (all10-200 µg/ml) were incubated for 24 hours. Results showed that all the drugs produced dose-dependent reductions in MMP activities. Glycyrrhiza glabra had lower cytotoxicity relative to

other agents. Diclofenac had the highest cytotoxicity. Due to low cytotoxicity and similar inhibitory activity against MMP, Matricaria aurea should be studied further for possible clinical utilization.

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CYTOTOXIC AND ANTI-INFLAMMATORY EFFECT OF VITAMIN E ON MATRIX METALOPROTEINASE ACTIVITY COMPARED TO STEROIDAL AND NONSTEROIDAL COMPOUNDS USING FIBROSARCOMA CELL LINE CULTURES

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The function of matrix metalloproteinase (MMP) in physiology of various inflammatory diseases such as rheumatoid arthritis and malignant tumors has been established. The aim of the present study was to compare the cytotoxic and anti-inflammatory effects of vitamin E with commonly used steroidal and nonsteroidal anti-inflammatory agents. For this purpose, zymographic and cellular cytotoxicity methods were employed, using fibrobsarcoma cell line culture (Whie 164). Vitamin E, diclofenac, dexametheasone and piroxicame (all10-200micog/ml) were incubated for 24 hours. Results showed that all the drugs, with exception of vitamin E produced dose-dependent reduction in MMP activities. In this study vitamin E had highest cytotoxicity relative to other agents used in this study. Due to high cytotoxicity of vitamin E, further studies towards understanding the underlying mechanism of its action is needed.

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EFFECT OF VITAMINS A,E,C AND OMEGA-3 FATTY ACIDS SUPPLEMENTATION ON THE LEVEL OF PARAOXONASE AND ARYLESTERASE ACTIVITIES IN STREPTOZOTOCIN INDUCED DIABETIC RATS: INVESTIGATION OF HEART, LIVER AND ERYTHROCYTES

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Objective: The aims of this study are to investigate the effect of supplementation with vitamins A, E and C and ω-3 fatty acids on paraoxonase and arylesterase activities in streptozotocin (STZ) induced diabetic rats. Materials and Methods: 64 male Wistar rats weighing 250g were divided into four groups as normal control, diabetic control, diabetic with vitamin A, E and C supplementation and diabetic with ω-3 fatty acids supplementation. After four weeks the rats were anesthetized and paraoxonase and arylesterase activitie were investigated in blood samples, and liver and heart homogenates. Results: In diabetic rats heart and liver arylesterase ($P<0.01$) activities were significantly less than normal control rats. Vitamin A, E and C supplementation and ω-3 fatty acids supplementation significantly increased liver arylesterase ($P<0.05$). No significant change was observed in other samples. Conclusion: Supplementation of Vitamin A, E and C and ω-3 fatty acids was found to increase liver arylesterase activity in diabetic rats and they can be valuable candidates in the treatment of the complications of diabetes.

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EFFECT OF VITAMINS A, E, C AND OMEGA-3 FATTY ACIDS SUPPLEMENTATION ON THE LEVEL OF LIPID PEROXIDATION IN STREPTOZOTOCIN INDUCED DIABETIC RATS: INVESTIGATION OF HEART, LIVER AND PLASMA

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Objective: The present study was designed to assess the effect of supplementation with vitamins A, E and C and ω-3 fatty acids on lipid peroxidation in streptozotocin (STZ) induced diabetic rats. Materials and Methods: 64 male Wistar rats weighting 250g were divided into four groups as normal control, diabetic control, diabetic with vitamin A, E and C supplementation and diabetic with ω-3 fatty acids supplementation. After four weeks treatment, the rats were anesthetized and malondialdehyde (MDA) levels were investigated in blood samples, liver and heart homogenate. Results: In diabetic rats MDA level in plasma, liver and heart was significantly more elevated than normal control rats ($P< 0.05$). Vitamin A, E and C supplementation caused significant decrease in plasma, liver and heart MDA ($P<0.05$). A significant decrease in heart MDA ($P<0.05$) was observed in diabetic rats with ω-3 fatty acids supplementation. Conclusion: Supplementation of Vitamin A, E and C and ω-3 fatty acids was found to decrease lipid peroxidation to some extent in diabetic rats and they can be valuable candidates in the treatment of the complications of diabetes.

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COMPARATIVE STUDY OF ANGIOTENSIN CONVERTING ENZYME (ACE) ACTIVITY, LEVELS OF LIPIDS AND APOLIPOPROTEINS IN PATIENTS WITH CORONARY ARTERY DISEASE AND NORMAL SUBJECTS

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The most important cause of mortality in developed and developing countries is coronary artery disease (CAD). Since,

CAD is a multifactorial disease, thus, we evaluated serum angiotensin converting enzyme (ACE) activity, lipid profiles and some apolipoproteins in the study population. A population-based cross sectional study was conducted with two hundred subjects (94 controls and 106 patients). Patients with 50% angiographically proved coronary stenosis referred to Tehran Shahid Rajaii Heart Hospital were recruited according to a designed protocol. The amounts of TG, BMI, HDL-C, VLDL-C, Apo A1, Apo B100 and ACE activity were measured by enzymatic, precipitation and HPLC methods in two groups. The results showed significant differences in the serum ACE activity, HDL-C, TG, VLDL-C, APO A1 and BMI in patients as compared with controls. Furthermore, multiple linear regression analysis showed odds ratios for ACE activity, cholesterol, VLDL-C and HDL-C 1.08, 1.24, 1.05 and 0.68 respectively. Moreover, the ACE activity had no significant correlation with other studied factors. We concluded that the increase of serum ACE activity is probably an independent factor in developing of atherosclerotic process resulting in elevation of CAD incidence in the study population.

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ACTIVITIES OF ANTIOXIDANT ENZYMES, CATALASE AND GLUTATHIONE REDUCTASE IN RED BLOOD CELLS OF PATIENTS WITH CORONARY ARTERY DISEASE

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Free radical scavenging enzymes are an important part of body anti-oxidative system. The aims of this study were to evaluate the enzymatic activities of anti-oxidative enzymes of erythrocytes, catalase (CAT) and glutathione reductase (GR), that might be indicators of protective mechanisms involved in atherosclerosis and also to evaluate the serum lipids and lipoproteins which are thought to be correlated with these two anti oxidative enzymes. The study population consisted of 90 patients with angiographically proved coronary stenosis in surgery section of Tehran Rajaee cardiovascular center and 30 subjects without any coronary heart disease used as control. Glutathione reductase and catalase activities in erythrocytes were assayed. Patients did not have significantly decreased glutathione reductase activity compared to control subjects. However the catalase activity was significantly decreased in erythrocytes of the atherosclerotic patients. Atherosclerotic smoking patients had similar catalase activity compared to nonsmoking patients. However the glutathione reductase activity was lower in erythrocytes of the atherosclerotic smoking patients. No significant correlations were found between serum lipids and two anti-oxidative enzyme activities in patients and control subjects.

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EFFECT OF AQUEOUS SEED EXTRACT OF SECURIGERA SECURIDACA ON ERYTHROCYTE CATALASE ACTIVITY IN TYPE 1 DIABETIC RATS

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Background: Complications in diabetes mellitus are associated with free radicals and oxidative stress. The human body prevents these complications through antioxidant defense mechanisms. The aim of the study was to investigate the effect of aqueous seed extract of Securigera securidaca on erythrocyte catalase activity in type1 diabetic rats. Materials and Methods: In the present interventional study, thirty male Wistar rats were used. Animals were assigned to two groups including normal and diabetic rats (n=15 for each group). Each group was divided into control and experimental subgroups. The experimental subgroups daily received 100mg/kg and 200mg/kg of the extract intraperitoneally. After thirty days administration of the extract, blood was directly collected from the heart and erythrocyte catalase activity was assessed. Results: catalase activity in diabetic control group was significantly decreased (P=0.002). Furthermore, catalase activity in groups treated with doses of 100mg/kg and 200mg/kg of the extract was significantly different compared to control group (P=0.003). Conclusion: The aqueous seed extract of Securigera securidaca probably could be effective in decreasing diabetic complications through amplification of antioxidant response by altering catalase activity and consequently reducing oxidative stress.

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DIFFERENTIAL EFFECTS OF ACETAMINOPHEN AND AFLATOXIN B1 ON EXPRESSION OF LIVER CLASS-P GLUTATHIONE S-TRANSFERASE IN GROWING RATS

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Background: The superfamily ofGlutathione S-transferase enzymes play major roles in detoxification of xenobiotic compounds by catalyzing conjugation of xenobiotics to cellular glutathione (GSH). GST-Pi is an important class of GSTs that is expressed during early stage of life and during developmental stages. Its activity is relatively high during embryogenesis and immediately after birth and diminishes in normal adult rat liver. Objectives: To investigate the effects of hepatotoxic agents such as acetaminophen (APAP) and aflatoxin B1 (AFB1) on liver GST-Pi in rats during postnatal age. Methods: suckling rats (14±2 days old) were divided into groups (n=5) and treated either with APAP (250 or 450 mg/kg B.W) and AFB1 (3 mg/kg B.W). Livers were removed at different time intervals (2, 6, 12, 18 and 24 h) and processed for GST and GST-Pi activity at protein and mRNA levels (RT-PCR). Results: Administration of a single high dose of AFB1 (3 mg/kg BW) and APAP (450 mg/kg BW) to weanling rats caused a significant ($P< 0.05$) induction in total GST activity in developing rats. Based on Western blotting technique and GST-Pi specific mRNA amplification by RT-

PCR, GST-Pi protein level and its expression were not affected by APAP or AFB1. Conclusion: Despite the inducible effects of AFB1 and APAP on liver total GST activity, GST-Pi remained unaffected in response to the drugs at protein and mRNA levels.

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PURIFICATION AND PARTIAL CHARACTERIZATION OF PROCOAGULANT FACTOR (FACTOR V ACTIVATOR) FROM IRANIAN VIPERA LEBETINA

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The venom of many viperidae snakes appeared to contain proteins that affect blood coagulation. Vipera lebetina is one of the poisonous snakes of Iran. Its venom has been found to have potent effects on coagulation, through both pro-and anticoagulant mechanisms of blood coagulation. FactorV is a single-chain glycoprotein which plays an important role in the procoagulant and anticoagulant pathways. Thrombin activates factor V into factorVa. Factor V can also be activated by a wide variety of snake venoms. Our studies of the Iranian Vipera lebetina venom have demonstrated the existence of both coagulant and anticoagulant effects of the heamostasis system. In the absence of calcium, no coagulation of plasma was observed and the presence of calcium resulted in coagulating activity at low concentrations. Ffurther analysis by purified systems, it was demonstrated that this venom contains factor V activation activity. The factor V activator present in the venom was separated by gel filtration on Sephadex G-100 followed by ion exchange chromatography on DEAE-cellulose and affinity chromatography on heparin-agarose. It was shown that a single protein band is present in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under both reducing and non-reducing conditions. The molecular weight was estimated to be 29KDa by SDS-PAGE. This compound activated factor V to Va in the presence of calcium ions. It activated factor V in the presence of a low molecular weight substrate, BAEE (Nα-Benzoyl arginine ethylester). It also had weak amidase activity on S-2222 (benzoyl-Ile-Glu-Gly-Arg-p-nitroanilide). Its activity was inhibited by the serine proteinase inhibitor diisopropyl fluoro phosphate.

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ADRIAMYCIN-INDUCED ALTERATIONS IN SALMONELLA TYPHIMURIUM

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Adriamycin (AD) is a well-known anti-cancer agent displaying a broad-spectrum antitumor activity against both solid tumors and leukemias, but is also accompanied by severe toxic side effects. Being an anthracycline drug, AD is capable of generating superoxide and hydroxyl radicals, potential sources for toxic side effects. In this work, S. typhimirium cells were used as a model to study the effect of AD on superoxide dismutase (SOD) activity, as well as on other parameters. S. typhimurium cells were harvested after 24 h culture in the presence of 1, 5, 20, 50, 100, 150 and 300 mg/ml AD. Assays for SOD showed that the enzymatic activity decreased as AD concentration increased in the culture medium, going from 5.8 u/mg protein in the control to 1.6 u/mg protein in cells grown in the presence of 300 mg/ml AD. Besides SOD, all components of the respiratory chain (cyt.b560, b595, and d) were also inhibited by AD. The typical growth curve of S. typhimurium exhibited an increased lag period and a reduced logarithmic phase in the presence of AD. The same trends were observed when the amount of viable cells was recorded, with the additional feature that the number of viable cells in the stationary phase was constant in the control, but dropped in the presence of AD. In addition to growth inhibition, AD also caused alterations in the cell shape. Furthermore, AD interrupted the normal cell cycle and cell division leading to the appearance of skeins in the culture upon examination under the microscope.

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ADENOSINE DEAMINASE ANALYSIS IN SERA OF HEALTHY VOLUNTEERS AT PASTEUR INSTITUTE OF IRAN

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Adenosine deaminase (ADA; EC 3.5.4.4), a ubiquitous polymorphic enzyme, catalyzes the deamination of adenosine/deoxyadenosine to inosine/deoxyinosine. It consists of ADA1, ADA1+cp and ADA2 isoenzymes. ADA has received much attention as a valuable biochemical test in clinical laboratories. In this study, blood samples were obtained by venous puncture from volunteers (40 healthy men and women). Total serum ADA activity (tADA) was assessed by an automated kinetic method. Liberation of ammonia, a product of the transformation of adenosine to inosine, was monitored at 340 nm by coupling the reaction to the NADPH-linked reductive amination of α-ketoglutarate catalyzed by glutamate dehydrogenase. SDS-PAGE pattern of ADA1 was studied in serum and erythrocytes of normal subjects. ADA1 in erythrocytes showed a typical band with molecular weight of approximately 30 kDa on 10% and 12% gels stained with Coomassie Brilliant Blue. In contrast no such band was detected under similar conditions in serum, which might be explained by the presence of excess combining protein (CP) in serum. The mean tADA of healthy individuals was measured to be 14.9±2.76 IU/l. There was no correlation between ADA activity and blood type, age or sex of healthy donors. This research is going to be continued on patients with immunodeficiency diseases.

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AGE DEPENDENT CHANGES IN HEPATIC MICROSOMAL CYP450 1A1 IN RATS TREATED WITH ACETAMINOPHEN

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Introduction: Cytochrome P450 enzymes which among them CytP450 1A1 and CytP450 2E act during metabolism of acetaminophen, have an important role during phase I of toxin metabolism. The effect of acetaminophen in newborn has been the subject of several studies, but the effect of this compound in newborn and adult rats has not been investigated. Materials and methods: In this study, liver tissue and blood samples were collected from injected rats and the effect of acetaminophen on the former was measured by EROD, and their effect on the latter was measured by FRAP during different time-spans and in different doses. Results: It was shown that low doses of acetaminophen could not induced the activity of this enzyme, but by increasing the dose of acetaminophen up to 450 mg/kg BW, this enzyme was induced and the increase of induction was smaller in newborn in comparison with adult rats. FRAP in rats treated with acetaminophen increased and this increase was higher in newborn rats than adult rats. Conclusion: CYP450 1A1 shows increased activity by two different mechanisms and acetaminophen causes an increase in the activity of enzyme and also an increase in its expression at the gene level. The relationship between EROD and FRAP depends on the drug used.

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OXIDATIVE STRESS IN PATIENTS WITH HYPERTENSION

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It has been suggested that activation of vascular NADPH oxidase by Ang II is the source of ROS in hypertension. The objective of this study was to compare the activity of a series of plasma antioxidant enzymes i.e. glutathione reductase (GR), glutathione peroxidase (GPx) and gamma-glutamly tranferease (GGT) in hypertensive patients and control subjects. Hypertensive patients (n=58; male/famle ratio of 37/21) with a mean age of 57.5 ± 9.1 years and control subjects (n=89; male/female ratio of 77/12) with a mean age of 54.9 ± 10.6 years were recruited. Plasma GR, GPx and GGT activities were determined spectrophotometrically. Plasma GR activity was significantly higher in the patient group (38.7 ± 6.4 U/L) than in controls (35.8 ± 7.6 U/L, P = 0.018). Elevated GPx and GGT activities were noted in the patient group than in control subjects (135.4 ± 58.8 U/L vs 122.7 ± 40.1 U/L and 14.2 ± 9.2 U/L vs 11.8 ± 7.1 U/L, respectively) but the differences failed to reach statistical significance. Correlation was obtained between GR and GGT activities (r= 0125; p=0.008). These findings indicate that enhanced oxidative stress in patients with hypertension leads to compensatory increment of antioxidant enzyme activities. Increased oxidative stress may lead to tissue damage and development of CVD.

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PREVALENCE OF GLUCOSE 6-PHOSPHATE DEHYROGENASE DEFICIENCY IN THE MALE NEWBORNS IN LARESTAN HOSPITALS AND ITS RELATIONSHIP WITH NEONATAL HYPERBILIRUBINEMIA

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Introduction: Glucose 6-phosphate dehydrogenase deficiency (G6PD) is the most common human enzymopathy. G6PD deficiency is an X-linked inherited disease and approximately 200 million people are affected worldwide. G6PD catalyses the first step in pentose phosphate pathway to produce NADPH. NADPH plays an important role in oxidation - reduction reactions of hemoglobin and reduces H2O2 level. The G6PD variants with reduced activity produce favism or may cause hemolysis after ingestion of fava beans. The incidence of G6PD deficiency is most common in south and north of Iran .The aim of this study was to determine the prevalence of G6PD deficiency and its relation to neonatal hyperbilirubinemia. Materials & methods: This cross-sectional study was performed on 1345 umiblical cord blood. Enzyme activity was measured with the method of fluorescent spot test (FST) divided into categories of sufficient (S), partially deficient (PD) and deficient (D). D and PD were considered as G6PD deficiency. Data were analyzed by SPSS. Among 1345 male neonates, 10 cases (0.7%) had a total bilirubin of over 18mg/dl, 184cases (13.7%) had a total bilirubin between 10-18 mg/dl and others had a total bilirubin under 10mg/dl. Out of 1345 male neonates, 192 cases (14.3%) had deficient, 12cases (0.9%) had partially deficient and only 3cases (0.22%) were G6PD deficient with a total bilirubin concentration over 18mg/dl. There was no statistically significant correlation between G6PD and neonatal hyperbilirubinemia. Conclusion: We suggest using the screening umbilical cord blood test on the male neonate to diagnose the G6PD deficiency and to promote the primary health care quality reduce the social and economic problems of the society.

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THE ANALYSIS OF ADENOSINE DEAMINASE ACTIVITY IN THE PRESENCE OF AMPICILLINL AT PHYSIOLOGICAL AND PATHOLOGICAL TEMPERATURES.

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Adenosine deaminase (ADA, EC 3.5.4.4) is a metalloenzyme which catalyses the hydrolysis of adenosine or deoxyadenosine to inosine or deoxyinosine and ammonia. In humans the highest ADA activity is found in thymus and other lymphoid tissues and the lowest in erythrocytes. ADA exists in different species from bacteria and plants to humans and the amino acid sequence is highly conserved specially in the active site. ADA is a very important agent for development and differentiation of immune system. In this study we investigated the ampicillin effect on the ADA activity in the range of adenosine concentrations from 14 to 450 nM at physiological and pathological temperatures. Our data showed that oral administration of 500 mg Ampicillin activates the enzyme in plasma and adipose tissue while this effect is decreased by the injection of the same concentration of Ampicillin at physiological temperature. The drug effect on the enzyme activity differs at pathological temperatures and the activation level is decreased. It could be concluded that the oral administration of Ampicillin activates ADA at 42^{o} C less than 37^{o} C in cardiac plasma and adipose tissue and the injection of drug tends to inhibit ADA especially in > 0.1 μM of adenosine. The docking analysis of Ampicillin binding sites on the enzyme showed 3 different sites, some of which are activatory sites and some are inhibitory sites for Ampicillin. Therefore it can be expressed that ampicillin have activatory and inhibitory regulatory effect on the ADA activity depending on the drug concentration.

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EVALUATION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE STATUS OF NEONATES IN SEMNAN CITY FOR A PERIOD OF ONE YEAR.

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Glucose-6-phosphate dehydrogenase (G6PD) is an enzyme in the pentose phosphate pathway, a metabolic pathway that supplies reducing energy to cells (most notably erythrocytes) by maintaining the level of the co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH). The NADPH in turn maintains the level of glutathione in these cells helping to protect the red blood cells against oxidative stress damage. G6PD deficiency (an X-linked recessive hereditary disease) is an inherited condition in which the body doesn't have enough of the enzyme G6PD. This deficiency can cause hemolytic anemia, usually after exposure to certain medications, foods, or even infections. G6PD deficiency is said to be the most common enzyme deficiency disease in the world, affecting approximately 400,000,000 people globally. Fava beans contain high levels of vicine, divicine, convicine and isouramil and all these compounds are oxidants. The classic reaction to consumption of fava beans has led to the commonly used term favism. It occurs more commonly in children than adults. The goal of G6PD activity testing is to detect G6PD deficiency and to determine its potential severity. It is especially important to screen newborns that are likely to have G6PD deficiency to ensure that G6PD-deficient babies woul not have to be subjected to any of the triggers of hemolytic anemia. Beutler fluorescent spot test is a direct test for G6PD which has largely replaced an older test (the Motulsky dye-decolouration test). The Beutler fluorescent spot test is a rapid and inexpensive test that visually identifies NADPH produced by G6PD under ultraviolet light. This study was conducted on 1849 newborns. There were 53.86% (996) males and 46.13% (853) females. In our one year study, there were 98.26% negative and 29 positive cases with 27 males and 2 females (1.74%). G6PD deficiency caused some problems such as shortness of breath, rapid heart rate, yellow skin color (jaundice), dark urine, and enlarged spleen. In most cases, the risks can be minimized by avoiding oxidant drugs and chemicals, and foods containing fava beans. With suitable treatment and good dietary control, the potential effects of G6PD deficiency on development are minimized in newborns. The result of this data should be shared with the parent and related organizations for controlling the problem.

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EFFECT OF VITAMINS A, E, C AND OMEGA-3 FATTY ACIDS SUPPLEMENTATION ON THE LEVEL OF CATALASE AND SUPEROXIDE DISMUTASE IN STREPTOZOTOCIN INDUCED DIABETIC RATS: INVESTIGATION OF HEART, LIVER AND ERYTHROCYTES

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Objective: The current study was designed to assess the effect of supplementation with vitamins A, E and C and ω-3 fatty acids on catalase and superoxide dismutase activity in streptozotocin (STZ) induced diabetic rats. Materials and Methods: 64 male Wistar rats weighing 250g were divided into four groups as normal control, diabetic control, diabetic with vitamin A, E and C supplementation and diabetic with ω-3 fatty acids supplementation. After four weeks the rats were anesthetized and catalase and superoxide dismutase activity was investigated in blood samples, and liver and heart homogenates. Results: In diabetic rats heart SOD ($P<0.001$) and heart and liver CAT ($P<0.001$) activities were significantly less than normal control rats. Vitamin A, E and C supplementation significantly increased heart CAT ($P=0.05$). No significant change was observed in diabetic rats with ω-3 fatty acids supplementation. Conclusion: Supplementation of Vitamin A, E and C and ω-3 fatty acids was found to increase heart CAT activity in diabetic rats and they can be valuable candidates in the treatment of the complications of diabetes.

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SERUM PARAOXONASE-1 ACTIVITY, LIPID PROFILE, MALONDIALDEHYE AND TOTAL ANTIOXIDANT STATUS AND THEIR CORRELATIONS IN HYPERLIPIDEMIC DIABETIC PATIENTS

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Diabetes mellitus results in oxidative stress with diabetic complications such as lipid abnormalities, protein glycation, lipid peroxidation and increased production of free radicals. Paraoxonase-1(PON-1) is a serum enzyme with an antioxidant function protecting low density lipoproteins (LDL) from oxidative modification. The aim of our study was measurement of enzyme activity, malondialdehyde (MDA) level and total antioxidant status (TAS) in hyperlipidemic diabetic patients. Twenty five hyperlipidemic diabetic patients (11 females and 14 males) and seventy five healthy subjects (36 females and 39 males) were included in our study. PON1 activity was measured using paraoxone and phenylacetate as substrates. MDA measurement was made by thiobarbituric acid and TAS was determined by Randox company kit. Serum paraoxonase activity was significantly reduced in diabetic patients ($p<0.05$). Also, MDA level increased (0.75 ± 0.23 µmol/L) and TAS decreased (1.12 ± 0.32 mmol/L) in diabetic patients. Decreased activity of PON1 in diabetic group may be due to low HDL concentration and its biochemical modification (glycation). This factor is a useful indicator of coronary heart disease (CHD) in diabetic patients.

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THE ANALYSIS OF ADENOSINE DEAMINASE ACTIVITY IN THE PRESENCE OF ETHANOL AT PHYSIOLOGICAL AND PATHOLOGICAL TEMPERATURES

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Adenosine deaminase (ADA, adenosine aminohydrolase, EC 3.5.4.4) is a metalloenzyme that catalyses the irreversible hydrolysis of adenosine or deoxyadenosine to inosine or deoxy inosine and ammonia. The enzyme plays an important biological role in the metabolism of purine nucleotides and is essential for the proliferation and differentiation of lymphoid cells, particularly T cells, and maturation of monocytes to macrophages and shows its highest activity in T lymphocytes. Since adenosine is an important neuromodulator and plays a crucial role in signal transduction, this enzyme asserts its physiological role by regulation of adenosine concentration. In this study the effect of several concentrations of alcohol has been studied on the ADA activity at physiological and pathological temperatures in vitro. The results showed that the ADA activity in the presence of alcohol is different in 37°C and 42° C. The most effective concentrations of alcohol on the ADA activity were 20 µg/dl and 10 µg/dl at 37 and 42°C respectively. It is suggested that the adenosine concentration affects ADA response to alcohol and regulates the ADA activity. Furthermore the response of ADA to alcohol in different tissues that have different concentrations of adenosine is not the same. We suggest that plasma, adipose and cardiac tissues ADA are responsive to ethanol at 37° C and 42°C.

p-66
SERUM ADENOSINE DEAMINASE ACTIVITY IN TUBERCULOSIS AND NON-TUBERCULOSIS RESPIRATORY DISEASE

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Tuberculosis is one of the main causes of death in the world and it is a major health problem in Sistan and Balouchistan province of Iran. Adenosine deaminase (ADA) is a polymorphic enzyme that is involved in purine metabolism. It catalyses the deamination of adenosine and deoxyadenosine to inosine and deoxyinosine, respectively. This study was done to determine serum adenosine deaminase (ADA) activity in pulmonary tuberculosis, non-tuberculosis pulmonary disease and healthy subjects. The serum ADA levels, were significantly higher ($P<0.001$) in pulmonary tuberculosis (19.78 ± 7.09 U/L) as well as non-tuberculosis respiratory disease (14.78 ± 4.65 U/L) than healthy controls (10.02 ± 1.99 U/L). The optimum cut-off level of ADA was found to be 16.5 U/L in distinguishing tuberculosis from non-tuberculosis respiratory disease using ROC curve. Sensitivity, specificity, positive predictive value and negative predictive value were 71.7%, 63.3%, 42.8% and 84.4%, respectively. The results indicate that serum ADA activity is not a useful test to differentiate pulmonary tuberculosis from other respiratory diseases.

p-67
THE PEROXIDASE ACTIVITY OF HAPTOGLOBIN 1-1 PHENOTYPE - HEMOGLOBIN COMPLEX IN THE PRESENCE OF AMPICILLIN AND COAMOXICLAV AT PHYSIOLOGIC AND PATHOLOGIC TEMPERATURES UNDER IN VITRO CONDITIONS

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Haptoglobin is a mammalian serum glycoprotein of the alpha2 – globulin fraction. Haptoglobin is hemoglobin – binding antioxidant showing a genetic polymorphism with three types: Hp 1-1, Hp 2-1 and Hp 2-2. Haptoglobin can interact with

hemoglobin strongly and increase its peroxidase properties. Hp appears to conserve and foster the recycling of heme – iron by forming an essentially irreversible but non- covalent complex with Hb which has been released from erythrocytes into the plasma by lysis. Hydrogen peroxide is used as the oxidizing substrate in the peroxidase reaction. Guaiacol is the hydrogen donor. The formation of tetraguaiacol during the reaction is followed spectrophotometerically. The analysis of interaction between haptoglobin 1-1 and hemoglobin has been studied in the presence of ampicillin and coamoxiclav at physiologic and pathologic temperatures. The kinetic parameters (Vmax and Km) were calculated for both drugs at 37 ^{o}C and 42^{o}C. The results showed that Vmax and Km decreased in the presence of both drugs under the mentioned conditions. It could be concluded that ampicillin and coamoxiclav are uncompetitive inhibitors for peroxidase enzymatic reaction of haptoglobin – hemoglobin interaction. Therefore the risk of oxidant radical formation could be increased by this reaction in the presence of theses drugs. Therefore, the administration of these drugs could increase the tissue damages due to oxidant radicals for some of the patients with Hp 1-1 phenotype who have diabetic retinopathy, diabetic nephropathy and cardiovascular disease.

p-68
THE PEROXIDASE ACTIVITY OF HAPTOGLOBIN 2-2 PHENOTYPE - HEMOGLOBIN COMPLEX IN THE PRESENCE OF AMPICILLIN AND COAMOXICLAV AT PHYSIOLOGIC AND PATHOLOGIC TEMPERATURES UNDER IN VITRO CONDITION

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Haptoglobin (Hp) is a plasma glycoprotein that has been identified as a component of α2- globulins. The Hp molecule effectively recycles heme – iron by the formation of an irreversible, high affinity complex with hemoglobin (Hb) that has been released into the blood stream by red cell hemolysis. The binding of haptoglobin is one of the strongest known non-covalent interactions in biology. Essentially three major phenotypic forms of human Hp, designated as Hp 1 -1, Hp 2-1 and Hp 2-2, have been isolated from human serum. The interaction of Hp- Hb can increase the peroxidase enzymatic properties of haptoglubin. Hydrogen peroxide is used as the oxidizing substrate in the peroxidase reaction. Guaiacol is the hydrogen donor. The formation of tetraguaiacol during the reaction is followed spectrophotometerically. The analysis of interaction between haptoglobin 2-2 and hemoglobin has been studied in the presence of ampicillin and coamoxiclav at physiologic and pathologic temperatures. The kinetic parameters (Vmax and Km) were calculated for both drugs at 37^{o} C and 42^{o} C. The results showed that Vmax and Km increase in the presence of both drugs under the mentioned conditions. It could be concluded that ampicillin and coamoxiclav are activators of peroxidase enzymatic reaction of haptoglobin – hemoglobin interaction.The administration of these drugs for some of the patients with Hp 2-2 phenotype with cardiovascular disease decreases the tisuue damage due to oxidant radicals by activating the peroxidase properties of Hp-Hb complex.

p-69
HEALING EFFECT OF CHICORY IN EXPERIMENTAL HEPATOTOXICITY INDUCED BY PARACETAMOL IN BROILERS

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It has been reported that Chicory extract stimulates the production of bile and empties the gall bladder of laboratory animals and human, but this effect has not been reported in chicken. This study was conducted on 63 chicken, 21 days old with approximately the same weight. They were divided randomly into three groups of 21 chickens each: - control group, fed with normal diet and normal water during the period.; a second group injected with 0.5g Paracetamol / kg body weight (IP) in 24 day of life;.third group receiving 1% alcohol extract of Chicory through their water from 21 till 34 day of life injected with 0.5g Paracetamol / kg body weight (IP) on the 24th day of life. ALT, AST, LDH, CPK, GGT, uric acid and creatinine were measured in 25th, 27th and 34th day sera samples. First and third days after Paracetamol injection, the mean serum AST, GGT and CPK activities and serum concentration of uric acid in the second group showed significant increases compared with control and the third groups. The second group also showed significant loss of weight compared with the control and the third group. It proves that Paracetamol is a hepatotoxin and probably nephrotoxin for broilers and Chicory extract has hepatoprotective effects.

p-70
DETERMINATION OF THE SUSCEPTIBILITY OF ERYTHROCYTE CATALASE TO SUICIDE INACTIVATION IN ACUTE LYMPHOBLASTIC LEUKEMIA CASES AND ITS COMPARISON WITH RELATED CONTROLS

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Catalase (CAT) is inactivated by its suicide substrate, hydrogen peroxide. The rate constant of this inactivation process, ki, is a measure of the susceptibility of CAT to inactivation during catalysis. So, elevation in ki value can be treated as an indicator of probability of defect in the biological role of this antioxidant enzyme. The present study is focused on measurement of ki of CAT in hemolysates obtained from acute lymphoblastic leukemia (ALL) patients The mean obtained for ki value of CAT in 75 ALL patients was 13.2 (SD = 0.5) $M^{-1}min^{-1}$ at 37^{o}C, and pH 7.0. It was found that the measured ki is spread in a range that is not wider than that of

experimental errors on an individual specimen. Thus, all specimens of ALL patients exhibited the same value of ki. The comparison of ki values related to the case and control groups revealed that there is not a significant difference between the ki corresponding to ALL patients with that of controls ($p > 0.1$). It was concluded that (i) the susceptibility of CAT to inactivation during its catalytic activity is not a significant cause (if any) of ALL. (ii) Among our 75 ALL patients we were not able to find a case that his/ her RBC contains a CAT involved in an unusual inactivation process induced by the exposure of this enzyme with its suicide substrate.

p-71
DETECTION OF SERUM ANGIOTENSIN CONVERTING ENZYME ACTIVITY AND TOTAL ANTIOXIDANTS IN PATIENTS WITH CORONARY ARTERY DISEASE

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Background: Angiotensin –1 converting enzyme (ACE) is a dipeptidyl carboxypeptidase (EC: 3.4.15.1) that catalyzes the conversion of Angiotensin I to the potent vasoconstrictor Angiotensin II .ACE also catalyzes the degradation of bradykinin, a vasodilatory peptide. Angiotensin II is responsible for an increase in blood pressure and maintenance of hypertension through the stimulation of oxidative stress. In this study, the association between coronary artery disease (CAD), ACE activity, serum antioxidant status and ascorbic acid (vitamin C) were investigated which may benefit patients with coronary artery disease. Methods: A group of 67 male patients with angiographically defined coronary artery disease (CAD) and 57 age and sex-matched normal control subjects were studied. ACE activity, total antioxidant and ascorbic acid were also compared between 57 normal male controls and 35 normal females. The activity of angiotensin-converting enzyme (ACE) was determined by the reversed-phase high performance liquid chromatography (HPLC) to separate and quantify hippuryl- histidyl-leucine (HHL) and hippuric acid (HA).We used ferric reducing ability of plasma (FRAP Assay) as a measure of antioxidant power. Serum ascorbic acid was determined photometrically. Results: There were significant differences in ACE activity, antioxidant power and ascorbic acid levels between CAD cases and normal controls. ACE activity and total antioxidant power did not differ between sexes in normal subjects but ascorbic acid was significantly higher in females. Conclusion: The present study demonstrates increased ACE activity in patients with coronary artery diseaseand this increase is not sex related.

p-72
COMPARATIVE STUDIES ON THE CYANIDE METABOLIZING ENZYME (RHODANESE) IN TISSUES OF HUMAN AND ANIMALS

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The enzyme Rhodanese (thiosulfate:cyanide sulfurtransferase)plays a central role in cyanide detoxification in living organisms.The purpose of this study was to measure and compare rhodanese in selected tissues of human and domestic animals Tissue extracts were prepared from liquid nitrogen-frozen samples and rhodanese specific activity and activity per gram tissue was measured by determinatioin of enzymatically formed thiocyanate.The results showed that in humans the highest activity of rhodanese was present in kidney, followed by liver, brain, lung, muscle and stomach. Other tissues studied did not show significant rhodanese activity. Human liver contains lower rhodanese activity as compared with ruminants and nonruminants, except for dog which has comparable hepatic activity with humans. Human kidney contains significant activity and the value is higher than those of camels, pigs, dogs, and chickens and is comparable with those of goats.The result of this study might indicate the involvement of rhodanese in cyanide detoxification in tissues which might be more exposed to cyanide due to higher blood supply to these tissues.On the other hand, rhodanese might perform other functions in human organs which are specific to these tissues.

p-73
ANTIDIABETIC EFFECTS OF MELATONIN IN STREPTOZOCIN- INDUCED DIABETIC RATS

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Diabetes mellitus is a serious metabolic disorder associated with many functional complications. The present study was undertaken to better understand the mechanism of action of melatonin (MLT) against streotozotocin (STZ) - induced hyperglycemia in rat. Glucose tolerance tests, plasma triglycerides (TG) and cholesterol (CHO) and activity of hepatic glucokinase (GK), hexokinase (HK) and glucose-6-phosphate dehydrogenase (G6PD) were determined. In order to determine biochemical parameters three random groups of 8 male rats were divided into the healthy control group, a group of STZ-induced diabetic rats, and a group of diabetic rats receiving melatonin .MLT was given orally (5 mg kg-1) for 15 consecutive days after STZ treatment (40 mg kg-1 i.v.). Also, in order to determine the activity of hepatic enzymes, another 3 groups of rats with the same conditions mentioned above were chosen. The plasma glucose, TG and CHO level of diabetic rats receiving MLT decreased significantly by 60.8, 45.3 and 53.6% respectively, and reached the level of glucose in healthy control group ($P<0.05$). Besides, administration of MLT to diabetic rats significantly corrected the glucose tolerance curves to that of normoglycemic animals. The specific activities of hepatic GK, HK and G6PD in diabetic control rats were significantly reduced compared to their normal controls. Administration of MLT increased the specific activities of the GK, HK and G6PD by 4.5, 2.7 times and 98% respectively, compared to diabetic rats ($P<0.05$). In conclusion, MLT probably induced its effects in terms of decreasing plasma glucose, TG and CHO level and increasing of hepatic enzyme synthesis by increasing insulin secretion.

p-74
EFFECT OF 250 MICROTESLA INTENSITY ELECTROMAGNETIC FIELD ON SERUM ALANINE TRANSAMINASE AND ASPARTATE TRANSAMINASE ACTIVITIES IN MICE

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The widespread use of electric power results in exposure of humans to low level 50/60 Hz electric and magnetic fields. There are some controversial reports on interaction between electromagnetic field (EMF) and biological systems. Alanine transaminase (ALT) is a cytoplasmic enzyme that catalyzes the reaction of alanine to pyruvate reversibly. Aspartate transaminase (AST) catalyzes the reaction of aspartate to oxaloactate reversibly. ALT and AST are nonspecific enzymes of plasma and their elevations are indications of cellular damage. ALT and AST activities rise especially in liver diseases. The aim of this investigation was to study effect of 250 microTesla intensity of low frequency EMF exposure on serum ALT and AST activities of mice. In this study a total of twenty four Albino mice were divided into four groups each of six animals. The treatment groups were exposed to 250 μT intensity EMF with frequencies of 25, 50 and 100 Hz for one hour per day during thirty days. The control group was in the similar situation without exposure to EMF. The blood samples were taken after thirty days. ALT activity was determined at 37oC by kinetic method based on the conversion of alanine to pyruvate and the action of lactate dehydrogenase on pyruvate and measurement of NADH consumption. AST activity was determined at 37oC by kinetic a method based on conversion of aspartate to oxaloactate coupled to the action of malate dehydrogenase and reduction of NADH. Absorbance changes were measured at 340 nm by Eppendorf ECOM-E 6125 spectrophotometer. The SPSS software was used to analyze data. Our studies demonstrated that EMF increased serum ALT and AST activities in the groups exposed to 25, 50 and 100 Hz in comparison with the control group. Significant differences ($p< 0.05$) were detected in ALT and AST activities between the exposed groups and the control. In this study the serum ALT and AST activities enhancements were observed in 25, 50 and 100 Hz EMF with 250 microTesla intensity which indicates the electromagnetic fields have side effects on liver.

p-75
CYTOCHROME C OXIDASE ACTIVITY ENHANCED IN WBC OF PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Objective & backgrounds: Chronic obstructive pulmonary disease (COPD), a lung disease, is characterized by decreased expiratory flow rates, increased pulmonary resistance and hyperinflation. Cytochrome C Oxidase (COX) is a key oxidative enzyme that modulates oxygen uptake and catalyzes the oxidation of reduced Cytochrome c by molecular oxygen. In vitro studies indicate that the activity of COX can be directly regulated by the presence of molecular oxygen. Thus, a better understanding of the role of COX in patients with COPD can provide an important link between the availability of oxygen to tissues and the regulation of oxygen uptake and energy production in these patients. Methods: For this purpose, we studied 42 patients (36 males, 6 females) with COPD under clinically stable conditions and 50 (42 males, 8 females) healthy sedentary volunteers of similar age. Whole blood was collected by venipuncture in sodium citrate tubes and WBCs separated by Ficoll according to standard protocol and lysed with microtube pestle homogenizer. The homogenates were centrifuged and the supernatants were used as a cell extract for COX activity determination. Aliquots of this were assayed for total protein content and COX activity. Analysis of COX activity was performed using COX assay kit. Absolute specific COX activity was normalized for total protein. Relative activities were calculated by dividing absolute specific COX activity by absolute specific citrate synthase activity. Results: mitochondrial COX activity and specific activity (absolute& relative) in WBCs were significantly increased in patients with COPD in comparison with control samples ($p< 0.05$). Conclusion: These results indicate that the activity of COX is increased in WBCs of patients with COPD but whether this is a primary or secondary change relevant to hypoxic conditions in these patients is not clear and needs further investigation.

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PROTECTIVE ROLES OF ANTIOXIDANT CO-SUPPLEMENTATION ON NEPHROTOXIC EFFECTS OF CYCLOSPORINE A.

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The immunosuppressive agent cyclosporine A (CsA) has been reported to exert nephrotoxic effects due to reactive oxygen species (ROS) formation. In this study, the role of an antioxidant co-supplementation of vitamin E (vit.E) and quercetin (Q) was investigated in CsA toxicity in rats. Male Sprague Dawley rats were divided into five groups: control, 25 mg/Kg/day CsA, 100 mg/Kg/day vit.E+CsA, 15mg/Kg/day Q+CsA and vit. E+Q+CsA. Renal function was assessed by examining blood urea nitrogen (BUN), and serum creatinine. Kidney lipid peroxidation as malondialdehyde (MDA), antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) were also measured. Histopathological examination of hematoxylin-eosin, PAS, Jones and Masson's trichrome stained sections of the kidneys were also performed. CsA increased levels of MDA, creatinine and BUN and decreased antioxidant enzyme activities. Q alone could elevate the activities of CAT, GPx and SOD in the kidneys of CsA-treated rats to the values observed for the CsA group. Although vit. E could, to some extent, raise the antioxidant enzyme activities of CsA-traeted animals; it could only normalize GPx activity. Co

administration of vit.E and Q significantly prevented ill-effects of CsA and attenuated renal morphological changes in CsA-treated rats. It is concluded that, most probably, Q in addition to its antioxidant property, could also donate H atoms to tocopheryl radicals and recycle vit.E for further activity.

p-77
PLATELET ACTIVE COMPONENTS FROM AGKISTRODON BLOMHOFFII USSURIENSIS SNAKE VENOM

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Introduction: Snake venoms have been reported to contain a number of platelet active components including enzymes and nonenzymatic proteins and peptides. Some of these factors induce platelet aggregation or activation, whereas others inhibit platelet aggregation. Methods: We developed different model systems to test the influence of proteins from the Agkistrodon blomhoffii ussuriensis snake venom on platelet activation and aggregation. The systems were both done in vitro using aggregometry and flow-cytometry analysis. Molecular masses and the purity of the proteins were detected by 2D-SDS-PAGE. Results: Monomeric disintegrin (Blomus-B), phospholipase A2 (Blopholipase), fibrino (geno) lityc (Blomulyse) and thrombin-like enzyme (Ancistron-Bu) were purified from Agkistrodon blomhoffii ussuriensis venom using affinity, ion-exchange and hydrophobic chromatography. The results showed that Blomus-B and PLA2 isolated from this venom activate platelets and strongly inhibit their ADP- and adrenalin-induced aggregation in distinct ways. Blomulyse does not activate platelets and has no effect on the aggregation stimulated by collagen, but on the other hand it does inhibit ADP and adrenalin-induced platelet aggregation. Ancistron-Bu activates washed human platelets but have no effect on the aggregation in the absence of fibrinogen. Conclusion: The obtained results make it possible to draw the conclusion that purified proteins can be used for the development of new antiplatelet agents and as instruments for detailed elaboration and deep investigation of proceses which proceed with platelet participation.

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"ANCISTRON–BU" - A NOVEL THROMBIN-LIKE ENZYME FROM AGKISTRODON BLOMHOFFII USSURIENSIS VENOM: CHARACTERIZATION AND AVAILABILITY IN CLINICAL PRACTICE

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Snake venoms are known to contain proteases which act upon different stages of blood coagulation. A number of these enzymes are named thrombin-like enzymes, according of their ability to interact directly with fibrinogen molecules, causing them to polymerize into fibrin. The fibrin monomers, produced by thrombin-like enzymes, are non-cross-linked. Consequently, such abnormal fibrins are easily dispersible and more susceptible to plasmin proteolysis than thrombin-induced fibrin. Therefore, after intravenous administration, thrombin-like enzymes can cause a rapid defibrinogenation. A novel fibrinogen-clotting enzyme (ancistron–Bu) has been purified from Agkistrodon blomhoffii ussuriensis venom using affinity, ion-exchange and hydrophobic chromatography. Ancistron–Bu, proved as homogeneous and showed molecular weights of approximately 13.5 and 27 kDa in reducing and non-reducing conditions. Its yield was 2% of total protein and its purity increased 80 fold. This enzyme showed specific fibrinogen-clotting activity equivalent to 175 NIH/mg. Unlike alpha-thrombin, ancistron–Bu split off fibrinopeptide A without releasing fibrinopeptide B from fibrinogen. The optimal pH range for the clotting activity of ancistron–Bu was 7.4- 8.0. The hydrolytic activity of ancistron–Bu is strongly inhibited by benzamidine, but heparin–antithrombin-III complex has a small effect on its catalytic properties. This enzyme did not activate factor XIII. Thrombin-specific substrate and protein C substrate were most susceptible to hydrolysis by ancistron–Bu. Ancistron time is a simple alternative to the thrombin time for rapid fibrinogen assay in samples containing heparin–antithrombin-III complex. The presence of fibrin degradation products, hypofibrinogenaemia and defects in fibrin polymerisation will prolong the ancistron time.

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PLASMINOGEN ACTIVATOR FROM AGKISTRODON HALYS VENOM

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At present time one of the most important clinical subjects concerning the proteins isolated from snake venoms belonging to the Crotalidae and Viperidae families is isolation and characterization of plasminogen activators. A novel plasminogen-activating proteinase (named "Ahh-32") has been isolated and purified to homogeneity from crude Agkistrodon halys halys venom using affinity and anion-exchange chromatography on Blue Sepharose FF and DEAE Sepharose FF, respectively. The characteristics of obtained protein were estimated by 2D-PAGE and analytical size exclusion chromatography. Interaction of "Ahh-32" with main components of haemostatic systems was investigated using chromogenic substrates and enzymes in PAGE. The concluded research show that purified enzyme consists of a single peptide chain with a molecular weight of 32 kDa and can convert free plasminogen into plasmin via an enzymatic reaction. The purity of the protein was about 99%. The "Ahh-32", catalyzing the hydrolysis of several p-nitroanilide substrates, neither activates nor degrades prothrombin, factor X or protein C and does not clot fibrinogen, nor demonstrates fibrino (geno) lytic activity in the absence of plasminogen. The activity of "Ahh-32" was inhibited by DFF and benzamidine. Besides, the enzyme influenced significantly the activation of plasminogen by streptokinase without effect on analogical process in case of usage of tissue plasminogen activator. Obtained results make possible to draw a conclusion that serine proteinase isolated from Agkistrodon halys halys venom is a novel plasminogen activator which can be used as an instrument in investigation of protein-protein interactions in

haemostasis systems and for the development of new cardiovascular therapeutic agents.

Hormonal Disorders

p-80
THE FIRST REPORT ON INSULIN RECEPTOR GENE MUTATIONS IN IRANIAN PATIENTS WITH NIDDM BY PCR – CSGE METHOD

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Mutation in the insulin receptor alters its binding to receptor resulting in insensitivity of the receptor to insulin in diabetic patients. The objective of our study was to screen the whole Insulin Receptore gene for mutations in type II diabetes (NIDDM) patients in Iran. Using Conformational Sensitive Gel Electrophoresis (CSGE) Exons 1-13 and 18-22 of 22 exons were analyzed. Blood samples were obtained from 60 type II diabetes who were selected based on defined including criteria and DNA was extracted from by sodium per chlorate method.18 PCR reactions for each individual exon of insulin receptor gene (except for Tyrosine Kinas related ones) were accomplished by 18 different pairs of primers designed specifically for each exon. PCR products were subjected to CSGE (each PCR product was heated up to 96 degree centigrade and then cooled down up to 68 degree centigrade, then the result was electerophoresd on Formamide/Ethylenglycol containing polyacryl amide gel) and were sequenced. 4 out of 18 exons displayed mutations: A missense mutation in exon 3 (Gly 227 Asp) and exon 8 (Thr 543 ser) a missense mutation (Arg 890 Pro) and a silent one in exon 9 at position 66 and in exon 13 (Asp 865). Comparing the results of 25 not affected normal volunteers (without any familial history for diabetes) showed that the mutation in exon No. 8 could be a SNP in Iranian population since the normal ones showed the mutation too, but the mutations of other 3 exons (3, 9, 13) were detected just in affected ones and not the normal population. These remark more study in greater normal population to confirm the results and relate the mutations to pathogenesis of diabetes in these patients. There are some case reports relating mutations in the same exons to the disease.

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INTERMEDIN/ADRENOMEDULLIN2 INDUCES CAMP ACCUMULATION IN DISSOCIATED RAT SPINAL CORD CELL CULTURE: EVIDENCE FOR A DISTINCT RECEPTOR SYSTEM

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Intermedin (IMD)/adrenomedullin-2 (AM2) has recently been isolated by two independent groups (Roh et al, JBC, 2004; Takei et al, FEBS Lett, 2004). This peptide has structural similarities to the calcitonin family of peptides which includes calcitonin gene-related peptide (CGRP), adrenomedullin (AM) and amylin. IMD/AM2 has been shown to bind to and activate CGRP and AM receptors, and we reported that CGRP and AM act through distinct receptors to induce cAMP accumulation in dispersed cells from embryonic rat spinal cord(Poyner et al, SFN Abstr 2004). We characterized here the affinity and efficacy of IMD/AM2 for these receptors in our model. IMD/AM2 inhibited 125ICGRP binding to embryonic spinal cord cells with a pIC50 of 9.43±0.16. Interestingly, IMD/AM2 competed for specific 125IAM binding in a biphasic manner with pEC50s of 9.03±0.22 and 6.45± 0.24, respectively. Cellular levels of cAMP were increased by IMD/AM2 (pEC50 7.48±0.3) when cells were exposed to this peptide for 10 min at 37°C. This effect was partially inhibited by the CGRP antagonist BIBN 4096 BS (pA2 6.67), the AM antagonist human AM22-52 (pA2 6.74) and the AM/CGRP antagonist CGRP8-37 (pA2 7.06). More interestingly, a highly significant effect of IM/AM2 on cAMP accumulation (pEC50 7.3±0.14) was observed even in the presence of mixture of saturating concentrations of BIBN 4096 BS, human AM22-52 and the amylin antagonist AC0187. These data provide evidence for the existence of a distinct receptor for IMD/AM2 in embryonic rat spinal cord cells. Supported by a CHIR grant.

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COMPARISON OF THE ASSOCIATIONS OF APOLIPOPROTEIN B (APO B) AND LDL-C WITH OTHER CARDIOVASCULAR RISK FACTORS IN METABOLIC SYNDROME PATRIENTS

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Some of the cardiovascular risk factors which have been identified include dyslipidemia, diabetes mellitus, hypertension, smoking and insulin resistance. Apolipoprotein B has been mentioned as the best marker of lipid profile. Furthermore, LDL-C is the major lipoprotein related factor that produces atherosclerosis but comparison between apo B and LDL-C has rarely been performed for prediction of cardiovascular events. 66 adult patients with metabolic syndrome were chosen among the patients refered to the endocrinology and cardiology clinics in Mashad. The following variables were studied: Blood pressure, Triglyceride, cholesterol, HDL, LDL and apo B. The patients were divided into four groups upon high or normal levels of LDL, and also high or normal levels of apo B. Among total 66 patients there were 11 men and 55 women in the range of 21-78 years old with the mean of 46/15±13/43 years. 15% of the patients of this study had normal LDL but high apo B. The correlations of apoB with cholesterol, TG, HDL, LDL were significant ($p<0.001$ for cholesterol and $P<0.05$ for the others) but LDL-C correlated with cholesterol and waist only ($P<0.001$ for cholesterol and $P<0.05$ for waist). In conclusion, apoB correlated with a wider array of cardiovascular risk

factors, in comparison with LDL- C, indicating, that apoB is a better predictor of cardiovascular events. Therefore, apo B measurement is suggested to be added to routine lipid profile tests, at least in metabolic syndrome patients. Keywords: Apolicprotein B (apoB),LDL-C, cardiovascular risk factors, metabolic syndrome.

p-83
PURIFICATION OF BLOOD FREE LEPTIN AND ITS CORRELATION WITH INSULIN IN OBESE AND NON-OBESE DIABETES PATIENTS

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Leptin is a fat derived tissue hormone which has a central role in body energy regulation and metabolism. This hormone is present in free and bound form in blood. Blood levels of free leptin change in fasting, obesity and some other conditions. There is limited information about the correlation between free leptin and insulin. In this study we purified free leptin and analyzed its correlation with insulin and insulin resistance. Our studu group comprised of 30 obese diabetes BMI>30kg/m2, age:50±20) and 30 non-obese diabetes (BMI<25mg/m2,age 50±20). Free leptine was purified by Gel filtration chromatography using sephadex G-100. Samples were injected into column and all fractions were collected by fraction collector. The free and bound leptin forms in fractions were analysed by a sensitive ELISA method.The mean levels of free leptin in obese patients (5.56±4.2 ng/dl) was significantly (P<0.5) higher than those in non-obese diabetic patients (0.16 ±0.1 ng/dl). The ratio of free leptin to total leptin in obese diabetes patients was three times higher than non-obese patients (0.27±01 vs 0.03± 0.01 respectively, P<0.05). Free leptin correlated positively with insulin(r=0.39, P=0.002) and store fat(r=0.69, p=0.001). Also a positive correlation was observed between the ratio of free to total leptin with insulin(r=59, p=0.001), store fat(r=0.84, P=0.003) and insulin resistance (r= 0.39, P= 0.0010 in diabetic patients. This correlation was not significant in non diabetic individuals In conclusion; our results showed that free form of leptin can be purified by Gel- filtration. This form is more than 50 times higher in obese diabetics compare to non-obese patients. A positive correlation between free leptin, insulin and insulin resistance was observed.

p-84
STUDY OF ASSOCIATION BETWEEN SERUM ADIPONECTIN AND HDL - C AT DIABETIC TYPE 2 AND NONDIABETICS

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Adipose tissue has recently been shown to secrete bioactive proteins, called adipocytokines that may regulate glucose and lipid metabolism as well as vascular function. Adiponectin is an adipocytokine produced exclusively by adipocytes. Adiponectin expression is reduced in obesity, insulin resistance and type 2 diabetes, and its plasma concentration is inversely related to body weight, especially in visceral adiposity. Recent studies have indicated that adiponectin has anti-inflammatory, antiatherogenic and anti-diabetic properties. The ability of adiponectin to reduce insulin resistance in conjunction with its anti-inflammatory and anti-atherogenic properties makes this novel adipocytokine a promising therapeutic target. Also agents that enhance adiponectin secretion or action have potential for treatment of metabolic and vascular diseases. Adiponectin is also inversely associated with other traditional cardiovascular risk factors, such as blood pressure, low-density lipoprotein cholesterol and triglyceride levels and is positively related to high-density lipoprotein cholesterol (HDL- C) levels. Low levels of high-density lipoprotein (HDL) -cholesterol represent an independent cardiovascular risk factor (besides reduced physical activity).Furthermore, the mechanisms leading to decreased HDL -cholesterol levels are not known. We aimed to test the association between serum adiponectin and HDL-C in patients with diabetic type 2 and nodiabetics. 50 patients with type 2 diabetes and 50 controls were investigated for adiponectin serum levels and other lipid parameters. In type 2 diabetic subjects, even after correction for age and gender, serum levels of adiponectin positively correlated with HDL-C levels in an statistically significant manner (r = 0/451, Pvalue = 0/001). In conclusion, adiponectin seems to predict HDL-C levels in patients with diabetes mellitus type 2. Low levels of adiponectin are associated with low levels of HDL-cholesterol beside common metabolic risk factors and therefore represent an independent cardiovascular risk factor in type 2 diabetes. Thus, adiponectin is a potentially new drug target in the treatment of dyslipidaemia.

p-85
SERUM LEVELS OF RESISTIN IN OBESE NON-DIABETIC AND DIABETIC SUBJECTS

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Resistin is a novel hormone secreted by Adipocytes and belongs to the family of cysteine-rich C-terminal proteins.The role of resistin in human biology remains uncertain. The aim of this study was to compare the serum resistin levels in type II diabetes and non-diabetics with obesity and determine the correlation between resistin with biochemical and anthropometric indices. This study consisted of 35 diabetes and 35 non-diabetes subjects. Fasting lipid profile was measured by the enzymatic methods. NycoCard HbA1c Kit was used to measure HbA1c. Serum resistin and glucose levels were measured by enzyme immunoassay and glucose oxidase methods respectively. BMI was computed as weight in kilograms divide by height in meter squared (kg/m2). Our data showed that the mean of BMI and age were not significantly different between two groups. The mean serum levels of FPG, Triglyceride and HbA1c concentrations in obese type II diabetes were significantly higher than controls (P < 0.05). Serum resistin concentrations were not different between diabetic (7.16 ± 0.16 ng/ml) and non- diabetics (6.40 ± 0.61 ng/ml) but were significantly higher in women compared with men in both groups. Serum resistin

concentrations negatively correlated with systolic blood pressure, diastolic blood pressure and triglyceride whereas positively correlated with BMI and hip circumference in both groups. In conclusion, our findings suggest that the concentration of resistin is unlikely to be a major link between obesity and diabetes.

p-86

THE EFFECT OF FEEDING OF AERIAL PART OF SILYBUM MARIANUM ON BLOOD GLUCOSE AND LIPIDS IN DIABETIC RATS

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Use of medicinal plants for attenuation of hyperglycemia and restoration of lipids to normal level is clinically important. In this respect, Silybum marianum (SM) is a plant that can lower lipid peroxidation and lipids in an experimental model of hyperlipidemia. Therefore, we investigated the effect of chronic oral administration of this plant on serum levels of glucose, triglyceride, total cholesterol, HDL- cholesterol and LDL-cholesterol in diabetic rats. For this purpose, female rats (n = 36) were randomly divided into 4 groups, i.e. control, SM-treated control, diabetic, and SM-treated diabetic groups. The treatment groups received plant-mixed pelleted food (6.25%) for 4 weeks. Serum levels of glucose, triglyceride, total cholesterol, LDL- cholesterol and HDL- cholesterol were determined at the start day, 2nd, and 4th week of the study.. In diabetic group, serum levels of glucose at 2nd and 4th week after the experiment were increased in comparison to the levels glucose one week before the study ($p<0.001$). SM treatment of diabetic rats produced only a mild non-significant effect. Simililarly, triglyceride level in diabetic group increased 4 weeks after the experiment in comparison with related data one week before the start day ($P<0.05$). A significantly smaller level of triglyceride was observed in SM-treated diabetic rats ($p<0.05$). Furthermore, treated-diabetic group as compared to diabetic group had lower serum cholesterol level ($p<0.05$). On the other hand, HDL-cholesterol and LDL- cholesterol levels were significantly higher and lower ($p<0.05$) in SM-treated diabetic group as compared to untreated diabetic group respectively. Oral chronic administration of SM had no significant hypoglycemic effect and led to appropriate changes in blood lipid profile.

p-87

THE EFFECT OF CHRONIC FEEDING OF ALLIUM LATIFOLIUM ON BLOOD GLUCOSE AND LIPIDS IN DIABETIC RATS

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Use of medicinal plants for attenuation of hyperglycemia and restoration of lipids to normal level is clinically important. In this respect, there is evidence for antidiabetic potential of derivatives of Allium latifolium (AL). Therefore, the effect of chronic oral administration of this plant on serum levels of glucose, triglyceride, total cholesterol, HDL-cholestero and LDL-cholesterol in diabetic rats was investigated. For this purpose, male Wistar rats (n = 32) were randomly divided into 4 groups, i.e. control, AL-treated control, diabetic, and AL-treated diabetic groups. The treatment groups received plant-mixed pelleted food (6.25%) for 6 weeks. Serum glucose, triglyceride, total cholesterol, LDL- cholesterol and HDL-cholesterol levels were determined before the start day, and at 3rd and 6th weeks of the study. Serum glucose levels in diabetic group increased 6 weeks after the start of the experiment as compared to data related to one week before the start day ($p<0.001$). AL treatment of diabetic rats produced a significant hypoglycemic effect ($p<0.01$). In addition, triglyceride levels in diabetic group 6 weeks after the experiment were higher in comparison to those of one week before the start day ($P<0.05$). There was no significant change in this parameter in AL-treated diabetic rats. Likewise, a significant reduction in serum levels of cholesterol was observed in treated-diabetic group as compared to diabetic group ($p<0.05$). On the other hand, HDL- cholesterol and LDL- cholesterol levels were significantly higher ($p<0.05$) and lower ($p<0.01$) in AL-treated diabetic group as compared to untreated diabetic group respectively. In conclusion chronic oral administration of AL has a moderate but significant hypoglycemic effect and leads to appropriate changes in levels of total cholesterol, HDL-cholesterol and LDL-cholesterol in serum.

p-88

EFFECTS OF AQUEOUS - ALCOHOLIC EXTRACT OF TEUCRIUM POLIUM ON INSULIN SECRETION FROM ISOLATED RAT PANCREATIC ISLETS

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Introduction: Teucrium Polium (Labiatae) grows widespread throughout Iran and shows hypoglycemic effects. To examine the mechanism of this effect, we assessed the effect of aqueous-alcoholic extract of the plant on insulin secretion from isolated rat pancreatic islets. Upper parts of the plant (stem, flower, and leaf) have been grinded and extracted by incubating in 500 ml alcohol 50 o at 40 oC for 72 hours. Then the solvent was evaporated in vacuum and reconstituted in DMSO which was diluted with Krebs solution. For isolation of Islets, in each experiment, rats were anesthetized with thiopental. The pancreas tissues were dissected and digested with collagenase. The isolated islets were collected manually under a stereomicroscope. Isolated islets were pre-incubated in Kreb's buffer with 3mM for 30min and then incubated with 3mM or 10mM glucose with or without isobutyl-methylxanthine (IBMX) or the extract for one hour. Data showed that 10mM glucose stimulated insulin secretion. IBMX augmented glucose-induced insulin release (GIIR) dose-dependently. While 0.05% concentration of the extract did not change GIIR a significant decreased in GIIR was observed in a higher concentration (0.5%) of the extract. In conclusion, Teucrium Polium extract has not insulinotropic property. The mechanism of inhibitory effect of the extract in the concentration of 0.5% is not clear and may be due to the

toxic effects of the extract in high concentrations. The in vivo hypoglycemic effect of Teucrium Polium could probably be the result of changing the rate of glucose metabolism or increasing the sensitivity of peripheral tissues to insulin.

p-89
THE EFFECT OF ORAL ADMINISTRATION OF AERIAL PARTS OF MARRUBIUM VULGARE ON LDL- AND HDL-CHOLESTEROL LEVEL IN AN EXPERIMENTAL MODEL OF DIABETES MELLITUS IN RAT

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Due to anti-diabetic effect of Marrubium vulgare (MV), its hypolipidemic effect was investigated in an experimental model of insulin-dependent diabetes mellitus. For this purpose, male Wistar rats (n = 44) were randomly divided into 4 groups, i.e. control, treated control, diabetic, and treated diabetic groups. For induction of diabetes, streptozotocin (STZ; 60 mg/Kg; i.p.) was used at a single dose. Serum glucose level higher than 250 mg/dl was considered as diabetic. The treatment groups received oral administration of plant-mixed pelleted food (6.25%) for two months. Statistical analysis of the data showed that serum glucose level in diabetic group increases 4 and 8 weeks after the experiment as compared to data one week before the study ($P<0.001$) and Marrubium vulgare treatment of diabetic rats did not have any significant effect. In addition, levels of LDL- cholesterol and HDL-cholesterol increased ($P<0.05$) and decreased ($P<0.01$) in diabetic rats respectively, and MV treatment significantly reversed this condition ($p<0.05$).In conclusion, these results showed that oral administration of Marrubium vulgare in long-term could significantly improve inappropriate changes in serum lipids and this may reduce the consequent complications in diabetes mellitus.

P-90
EFFECT OF ALCL3 ON HYPER AND HYPOTHYROIDISM IN RATS

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Poisoning with aluminum can change function of some glands in human and animals. In this experimental study, effect of $AlCl_3$ was evaluated in normal, hyper and hypothyroid rats. The study was done in 6 groups of rats (6 rats in each group) as follows: saline (as control), $AlCl_3$, levothyroxin (for hyperthyroidism induction), methimazole (for hypothyroidism induction), $AlCl_3$ and levothyroxin, and $AlCl_3$ and methimazole were administrated for 10 days in group 1 to 6 respectively. Serum concentration of T3 and T4 hormones were measured by radioimmunoassay and their means were compared by ANOVA test. Serum concentrations of T3 and T4 were significantly increased in group 5 with comparison to group 3 ($p<0.05$). $AlCl_3$ did not change serum concentration of T3 and T4 in normal rats. Thus, $AlCl_3$ can potentiate hyperthyroidism but has no effects on hypothyroidism.

p-91
EVALUATION THE EFFECTS OF ECSTASY ON PITUITARY- GONAD AXIS IN RATS

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Ecstasy is the colloquial name used to describe the entactogen, 3, 4- methylenedioxymethamphetamine (MDMA). Hallucinogens are a class of drugs that cause an altered state of consciousness characterized by illusions and hallucinations. Ecstasy is normally sold as 'brand name' tablets, which are given identifiable features such as imprints and/or colors. Ecstasy is frequently abused especially by young people. MDMA acts as an indirect monoaminergic agonist, mainly influencing the serotonergic, dopaminergic and noradrenergic systems. Acute administration of MDMA produces hyperthermia, hyperactivity, piloerection, salivation, mydriasis, ataxia and empathy together with a variety of physiological symptoms. The aim of the present study was to obtain evidence for effects of MDMA on pituitary- gonad axis. In this study three different doses of MDMA (2, 5, and 10mg/kg of body weight) in Euro ecstasy tablet were given orally to the rats every day for 30 days. Serum levels of LH, FSH and testosterone were measured. Histopathology of testises were also studied. Our data showed that concentrations of these hormones and the number of sertoli, leydig, spermatogonia and primary spermatocytes cells decreased dose dependently as compared with the control group. In conclusion, consumption of ecstasy exerted significant effects on sexual hormones and gonad tissues. Therefore MDMA (ecstasy) may have toxic effects on testises and other organs of the consumers.

p-92
RELATIONSHIP OF SERUM ADIPPONECTIN WITH HBA1C AND HS-CRP IN TYPE II DIABETIC WOMEN

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Adiponectin is secreted by adipose tissue and play an important role in hyperglycemia and inflammatory mechanisms. The objective of this study was to compare the levels of adiponectin , CRP and HbA1c in diabetic and healthy women and determine the correlation coefficient between them in each studuy group. We designed case-control study to assess baseline adiponectin concenterations in diabeteic and healthy women. We used spearman coefficient to determine correlation between adiponectin with CRP, HbA1c and age. After adjusting for age and BMI adiponectin showed to be lower in diabetic women(7.29 ± 1.42) than healthy women(10.29 ± 1.93)($P<0.01$) and there was a negative correlation between adiponectin with CRP and/or HbA1c, but

we didn't find any significant correlation between adiponectin and age. The obtained data indicated that diabetic women had lower adiponectin levels as compared to healthy women. HbA1c as an indicator of glycemic control showed negative correlation with serum adiponectin. Adiponectin can have an important role in the pathogenesis of diabetes and may be an independent predicator of development of diabetes in women. This study suggested the antidiabeteic and antiinflamatory properties of adiponectin.

p-93
EVALUATION OF THE THYROID AND LEPTIN HORMONES IN DEPRESSED PATIENTS

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Depression disorder has been associated with changes in the hypothalamus-pituitary-thyroid (HPT) axis. On the other hand, central nerves system is the most important site of leptin action.. However, the severity of the changes reported have been at variance probably related to in or out patient status, the use of anti depressant medication and heterogeneity of depression .Because of the inconsistencies in the published data we sought to assess the changes in leptin and thyroid hormones and evaluate their relation to depression. We measured endocrine parameters of 60 depressed women and 60 age, sex and BMI matched controls. Patients were selected randomly by a psychologist and using Beck Depression Inventory. We measured leptin, TSH, T4, T3, T3 uptake and also calculated FTI. Data showed that serum concentrations of thyroid hormones were not significantly different in both groups. The serum concentration of leptin in depressed group was significantly higher than the control group ($p<0.05$). The serum concentration of leptin in individuals with different degrees of depression (moderate, sever, very sever) also showed significant differences ($p<0.001$). In conclusion, results of this study clearly showed that in spite of the serum concentration of thyroid hormone. Because of the equality of BMI in two groups of the study depression is related to leptin hormone.

p-94
GROWTH HORMONE BINDING PROTEIN (GH-BP) IN A FAMILY WITH GROWTH HORMONE INSENSITIVITY SYNDROME (GHIS)

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Following our studies on GH-BP in human serum, an investigation was performed in a family with GHIS. The aim of this study was the characterization of GH-BP in this family to find if there is abnormal GH-BP, which could be a sign of abnormal growth hormone receptor (GHR). Due to low level of GH-BP in serum, micro-affinity column was developed in our laboratory with biotinylated-GH to purify exclusively GH-BP in serum from patient, patient's parents, and control normal subjects (10 ml). Serum was treated with proteinase inhibitors and loaded on the affinity column. SDS-PAGE, silver staining and western blotting were used to characterize the purified proteins. Our results showed the existence of the 52 and 56 kDa GH-BP in normal serum, while only 52 kDa GH-BP was identified in the patient and the mother. The sizes of GH-BPs in patient's father were comparable with normal. The absence of detectable 56 kDa GH-BP could be considered as a positive indication for GHIS. The deficiency of 56 kDa GH-BP in the patient could be due to termination of down-regulation. The initial step in down-regulation is dimerization of two isoform receptors by one GH molecule. This event triggers signal transduction and rapid degradation of GHR which can produce GH-BPs. Therefore, a mutant GHR that could not activate GHR down-regulation is not able to produce the 56 kDa GH-BP. The C-terminal sequence of human GH-BP and the site of production are not clearly known; hence a molecular study in individuals needs further investigation.

p-95
SURVEY OF LEPTIN AND INSULIN RESISTANCE IN OBESE AND NON OBESE DIABETIC(TYPEII) PATIENTS

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Diabetes is a common metabolic disease. There is a disorder in glucose metabolism and cause resistance to insulin action. There are reports about interaction between leptin and insulin and about effect of leptin on glucose hemostasis. Leptin is a 16-KD peptide hormone which affects on hypothalamus and prepheral tissues and caused decreasing food intake and increasing expenditure. It regulates body weight and BMI. In this study we chose 2 groups of 30 obese and non-obese non insulin dependent diabetic patients who were admitted to the center of diabetic clinical research of yazd.The obese person with BMI>30 kg/m² as a sample and non-obese person with BMI<25kg/m²as a control. We omited any person who used insulin drug; The range of age was between 30-70. Patients' serums were analyzed to determine the total leptin and insulin levels with ELISA. Total leptin levels were higher in the group of obese subjects compared with the group of non obese subjects ($p<0.001$). Insulin resistance was higher in obese subjects than non obese subjects ($p<0.001$). The total leptin was positively correlated with insulin and BMI. In this study leptin and insulin resistance were seen in NID diabetic obese and were higher than NID diabetic non- obese person. A direct correlation was found between leptin, insulin, insulin resistance which causes higher insulin resistance. This can prove the effect of leptin and insulin on the glucose hemostasis.

p-96
CORRELATION OF GHRELIN AND RESISTIN IN DIABETIC PATIENTS

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Reduction in insulin production or insulin receptors leads to diabetes mellitus (DM). Newly discovered hormones such as Ghrelin and Resistin could also manipulate energy, glucose, and lipid homeostasis. To investigate the influence of this hormones in diabetes (type-I and type-II), we measured circulating level of fasting and postprandial glucose, hemoglobin A1C, Ghrelin, Resistin and blood pressure of 80 DM patients (equal number of each type) and 80 healthy age and sex match person who served as two appropriated control groups. Body mass index (BMI), homeostasis model assessment (HOMA) and quantitative insulin sensitivity check (QUICK) were also calculated. Analysis of data showed serum level of Resistin is not changed in diabetic patients while it is significantly age dependent in type-II DM patients. Furthermore, circulating level of Acylated- Ghrelin is age dependent and its production is significantly lower in diabetic patients than their respective control groups. Calculation of HOMA and QUICK were significantly different in diabetic patients. Regression analysis showed there are significant correlations between Resistin and insulin (type-II), Resistin and QUICK or HOMA in control (II). In conclusion, hyperglycemia may affect Ghrelin production and insulin sensitivity or resistance in DM patients while circulating Resistin may affect their QUICK or HOMA.

p-97

EVALUATION OF THYROID AUTOIMMUNE MARKERS IN HYPOTHYROID DISEASE IN SABZEVAR

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Introduction: Thyroid disorder is one of the most important problems in all of countries special in the Iran. Many investigators have shown that it has incidence of autoimmune disorder in the region with enough iodine in the world and our country. Most of these studies have shown anti thyroid antibody and in particular anti peroxides antibody (Tpo Ab) and so, anti microsomal antibody and finally anti thyroglobulin antibody especially important. Although the health care of Sabzevar has illustrated that the incidence of hypothyroid is aroused in this city. The proposal of this study will determine autoimmune marker in male and female in the Sabzevar. We hope the results of this study are providing facility in diagnosis and treatment and also attainable care. Method & Material: This study is cross sectional and 382 male and female is choice that have TSH > 5µiu/ml. After collection of serum sample, they were frozen and later used to measure the amount of TSH with RIA and TPO Ab & TG Ab & TM Ab with ELISA. Relevant data were analyzed using Pierson correlation test. Results: In the initial studies we have found that it is amount of Thyroid peroxides antibody 63.8 and Microsomal antibody 62.4 and also anti thyroglobulin antibody 35.2. It has shown the standard correlation between hypothyroid and markers. Finally,the result of this study revealed that the amount of markers in the females is higher than in the males.

p-98

A SURVEY ON THE PRESENCE ANTI-GAD IN TYPE 1 DIABETIC PATIENTS AND THEIR FIRST-DEGREE RELATIVES IN COMPARISON WITH HEALTHY INDIVIDUALS

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Introduction: Glutamic acid decarboxylase (GAD) catalyzes the conversion of glutamic acid to □-aminobutyric acid (GABA). GAD65 isozyme is present in the pancreatic ß-cells. In prediabetes period and during the ß-cell destruction, GAD is released as an autoantigen and anti-GAD autoantibodies appear in serum. Islet Cell Autoantibodies (ICAs) including anti-GAD are detectable in serum of diabetic patients up to 10 years of age before appearance of the diabetes symptoms. This is an important predictive marker for diagnosis of prediabetic patients, especially in the first-degree relatives of diabetic patients for genetic factors. Materials and methods: This survey is a case-control study with the purpose of detection of anti-GAD presence in sera of type 1 diabetic patients and their first-degree relatives and comparison with healthy individuals. Fifty type 1 diabetic patients with mean age of 12.24 ± 6.2 years and mean disease duration of 34.5 ± 8.4 months as well as 35 first-degree relatives and 50 normal individuals without familial diabetes were included in the study. All the individuals were chosen by random sampling method. The values of fasting blood sugar were determined in first-degree relatives and controls and all were found to be normal. The values of anti-GAD were determined by ELISA method . Results: Median of anti-GAD in case and control individuals was 28, (range: 5-2700) ng/ml and 2, (0-10) ng/ml, respectively. The anti-GAD titers were significantly higher in patients than in normal and relatives together ($P<0.0001$) Median value of anti-GAD in first-degree relatives was 7, (0-950) ng/ml. There was a significant statistical difference between anti-GAD titers in first-degree relatives and controls, ($P<0.01$). There was a significant difference between mean value of age and diabetes duration in anti-GAD + and anti-GAD – patients, ($P<0.0$ 5). There was a negative correlation between anti-GAD and age, diabetes duration, disease beginning age of patients, (r=-0.155, -0.158, -0.036), respectively. Conclusion: By increasing of anti-GAD in diabetic patients and their first-degree relatives we conclude that measurement of anti-GAD is a beneficial tool for detection and diagnosis of prediabetic and diabetic patients.

p-99

ROLE OF GLUCOSE, CHOLESTEROL AND FATTY ACIDS IN DISSOCIATION OF TESTOSTERONE FROM PURIFIED HUMAN SEX HORMONE BINDING GLUBOLIN IN VITRO

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Increased free testosterone due to bioavailability or production in obese and diabetic women leads to development of hirsutism. Sex hormone binding globulin (SHBG) is a plasma glycoprotein that binds sex steroids with high affinity and is reduces in obese hyperinsulinemic women, resulting in increased androgen bioavailability. The effect of glucose and lipid elevation in this condition on dissociation of testosterone from SHBG is unknown. In this study we purified human SHBG by means of acetone and ammonium sulfate precipitation, DEAE-Sephadex chromatography and gel filtration and studied the effect of high concentration of glucose, cholesterol, stearate, oleate, palmitate and arachidonate on the level of free testosterone in medium containing human purified SHBG at 37ºc for 5 days. Free and bound testosterones were measured by RIA. The degree of amino group's modification was determined by TNBS method. Results showed that free testosterone level significantly increased after exposure of SHBG to high concentration of glucose, cholesterol, stearate and palmitate in comparison with control ($p<0.05$). Glucose had the greatest effect on free testosterone elevation in medium. Free testosterone did not significantly change after exposing of SHBG to oleate and arachidonate ($p<0.05$). It is concluded that high levels of glucose, cholesterol and saturated fatty acids in serum of obese and hyperinsulinemic patients may result in elevation of free testosterone due to SHBG modification. This work was supported by the Shiraz university enterpreneurship center.

p-100

RELATIONSHIP BETWEEN IGF-I AND LEPTIN IN OVERWEIGHT AND OBESE TYPE II DIABETIC PATIENTS AND CONTROLS

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Diabetes is a common endocrine disease in human. In most type II diabetic patient's obesity and overweight are also serious health problems worldwide. A variety of endocrine factors including Leptin and IGF-I system have regulatory role in weight balance and body composition.. The aim of this study was to examine the relationship between Leptin and IGF-I in type II diabetics and controls. This was a case-control study and composed of 50 patients of type 2 diabetes aged 50.54±10.37 and 50 healthy controls aged 47.54±9.94. All subjects had a Body Mass Index of at least 25. The concentrations of FPG, IGF-I, HbA1C and IGFBP-3 were measured in both groups. FPG was measured by enzymatic glucose oxidize method and HbA1C was measured by low pressure cation exchange chromatography. Determination of Leptin, IGF-I, IGFBP-3 and insulin concentrations were carried out using immunoassay method. The $p< 0.05$ was considered as statistically significant. Our data showed that the mean value of BMI and age were not significantly different in both groups. The mean serum levels of IGF-I, Leptin, insulin, FPG and HbA1c concentrations in type II diabetics were significantly higher than controls ($p < 0.05$). In males the mean serum levels of Leptin were statistically lower than in females in both groups. There was a reverse and significant correlation between IGF-I / IGFBP-3 molar ratio with Leptin ($P<0.001$, $r =-0.3$). However, Leptin had a positive and significant correlation with insulin ($p<0.001$, $r=043$). A reverse correlation was observed between IGF-I / IGFBP-3 molar ratio and HbA1c ($r=-0.18$, $p< 005$). In conclusion, based on these findings it is speculated that serum level of IGF-I is influenced by age, and decreased by rising of Leptin and also Leptin secretion has been suggested to be modulated by insulin .Therefore, Leptin and IGF-I system could have regulatory functions in body composition and fat content particularly in the obese and overweight diabetic patients.

O-101

CORRELATION BETWEEN CELL DAMAGE IN GRAVES' DISEASE WITH THE LEVEL OF OXIDATIVE STRESS

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Selenium is present in thyrocytes and follicular tissue in the form of glutathione peroxidase, selenoprotein P, and thioredoxin reductases. These selenoenzyme families possess powerful antioxidant properties and form a complex defense system that protects thyrocytes from oxidative damage. Abundant amounts of extracellular glutathione peroxidase are present in thyroid tissue to act as an antioxidant defense system against significant amounts of hydrogen peroxide resulting from thyroid hormone production. One of the main effects of increased concentrations of thyroid hormones in Graves' disease is increased speed of the basal metabolism, accompanied by an increase in the total consumption of oxygen, which results in increased formation of reactive oxygen species (ROS) and other free radicals, or the occurrence of oxidative stress. The aim of this study was to investigate whether serum levels of Se may influence the outcome of Graves' disease (GD). We performed a descriptive, observational, cross-sectional study in the setting of endocrinology clinic .120 subjects were selected that none of them had other known endocrine diseases. To evaluate the thyroid function, thyroid stimulating hormone (TSH), thyroxine (T4), (T3),(T3RU), and FTI levels were measured in the sera of patients. Two arbitrary serum-TSH threshold levels ($TSH < 1.0$ and > 4.0 mU/L) were introduced in order to classify hyperthyroidism and hypothyroidism respectively, as well as euthyroid conditions ($1.0 < TSH < 4.0$ mU/L) and each patient was assigned to one of these groups. Se levels were determined at the first visit in our outpatient clinic were determine by Graphite Furnace Atomic Absorption Spectroscopy with Nickel as a matrix modifier for background correction (AA670 G-SHIMADZU) .Data analysis was performed by SPSS NO-13 saftware. The serum levels of selenium in hyperthyroid patients were lower than other groups and the degree of cell damage in Graves' disease is in direct correlation with the level of oxidative stress, which depends on the efficacy of the capacity of the antioxidant

defense of the organism. Our data indicate that high serum Se levels may influence the outcome of GD. This is important, as Se administration trials in GD, which are under discussion, need to be performed with Se supplementation at high dosages.

p-102
FEASIBILITY STUDY OF NEW CALIBRATORS FOR THYROID-STIMULATING HORMONE (TSH) IMMUNOPROCEDURES BASED ON REMODELING OF RECOMBINANT TSH TO MIMIC GLYCOFORMS CIRCULATING IN PATIENTS WITH THYROID DISORDERS

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Differences between the glycosylation patterns of a pituitary thyroid-stimulating hormone calibrator (pitTSH) and serum samples have been shown to be responsible for non-identical epitope expression and for introducing discrepancies in TSH measurements. We studied the feasibility of developing new candidate reference materials by remodeling recombinant TSH (recTSH) to generate potential mimics of serum TSH. Terminal sialylation and/or inner fucosylation of recTSH were remodeled by a combination of enzyme treatments followed (or not) by lentil lectin-Sepharose affinity chromatography. The resulting TSH preparations were screened for epitope similarity in 23 immunoassays mapping 3 antigenic clusters common to the pitTSH 2nd International Reference Preparation (IRP) and the recTSH 1st IRP and then challenged against a pool of 63 patients with increased serum TSH (>60 mIU/L). pitTSH was poorly correlated with serum TSH, with a mean (SD) slope of 2.124 (0.001), in contrast to recTSH [slope, 1.178 (0.056)]. Comparison of variably sialylated preparations with recTSH gave slopes of 0.860 (0.057) for desialylated TSH, 1.064 (0.057) for 2,3/6-oversialylated recTSH, and 0.953 (0.033) for 2,6-resialylated recTSH, indicating that TSH forms enriched in sialic acid closely resemble serum TSH. Further testing against serum TSH showed satisfactory agreement with both TSH preparations containing 2, 6-sialic acid [slopes, 1.064 (0.057) and 0.953 (0.033)], particularly in the absence of nonfucosylated forms [0.985 (0.044)]. In conclusions Glyco-engineered recTSH preparations enriched in sialic acid and inner fucose are promising candidates for future reference materials. These preparations may have advantages over existing preparations used for standardizing TSH measurements.

p-103
EFFECT OF DIETARY FLAXSEED ON ENDOGENOUS SEX HORMONES AND UTERINE WEIGHT IN OVARIECTOMIZED FEMALE RATS.

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Flaxseed is one of the richest sources of the mammalian lignans that is known to have estrogen agonist or antagonist activities. The purpose of this study was to investigate the effect of flaxseed supplementation on plasma sex hormone levels in ovariectomized female rats. Experiments were conducted on four groups of 10 –wk-female rats: those in group 1 were ovariectomized and fed basal diet (0% flaxseed); those in group 2 and 3 were ovariectomized and fed 5 %and 10% flaxseed diet ;and those in group 4 were sham-operated controls. Plasma samples were analyzed for estradiol and testosterone after two months of dietary intake of flaxseed. The plasma levels of estradiol and testosterone remained unchanged. Moreover, flaxseed feeding for two months did not exert significant effects on the whole body and uterine weights. In conclusion, our data show that the levels of sex hormones and uterine weight in the ovariectomized female rats are not affected by flaxseed supplement.

p-104
IMPORTANCE of INCREASEd BLOOD PRO-CALCITONIN IN RELATION TO 5-YEAR-OLD CHILDREN URINARY INFECTION

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PCT (procalcitonin) is the hormonally inactive propeptide of calcitonin with a molecular weight of 12.6 KD that is normally synthesized by C cells of the thyroid gland. In severe infections (bacteria, fungi, parasites) as well as in sepsis, serum levels of PCT may increase. The aim of this study was to investigate the importance of increased blood PCT in relation to five-year-old children urinary infection. Five-year-old children (n=84) with urinary infection were selected by case series descriptive study. Blood levels of PCT were measured by chemiluminescence method. When compared with normal subjects, mean PCT concentration in the blood of severe urinary infected patients was 28.34 ± 1.5 that was statistically significant ($P<0.05$). Mean blood levels of PCT in moderate urinary infection was 0.95 ± 0.7 that was not statistically significant ($P>0.05$). Mean blood PCT levels in mild urinary infection was 0.2 ± 0.06 that was not statistically significant ($P>0.05$). In this study, blood PCT levels correlated with urinary infection severity as blood PCT concentration significantly was increased in severe urinary infection, but not in mild and moderate urinary infections.

Inborn Error of Metabolism

p-105
OXIDATIVE STRESS AND ANTIOXIDANT STATUS IN BETA-THALASSEMIA MAJOR

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Because of continuous blood transfusions, thalassemia patients are subject to peroxidative tissue injury by the secondary iron overload. The aim of this study was to evaluate the extent of lipid peroxidation and antioxidant status of patients with beta-

thalassemia and compare the results with healthy subjects. In accordance, oxidant and antioxidant status were studied in 20 regularly transfused beta-thalassemia major patients who were compared with 20 sex and age-matched healthy subjects. Malondialdehyde (MDA) was analyzed to indicate the oxidative status, whereas superoxide dismutase (SOD), vitamin E, reduced glutathione (GSH), and catalase were measured to show antioxidant status of the children. The results showed that plasma MDA was significantly increased about 2-fold in thalassemia patients compared to healthy subjects. Plasma levels of vit E and GSH were lower in patients with beta-thalassemia major. Ferritin levels were positively correlated with the amount of MDA, as further evidence of the deleterious effects of high tissue iron levels. Plasma levels of vitamin E and GSH were inversely correlated with ferritin, suggesting a major consumption of these antioxidants under iron overload. When compared to the controls, elevation in SOD and reduction in catalase activity was not significant .Our findings confirm the peroxidative status generated by iron overload in beta-thalassemia major patients and showed that reduction of antioxidant capacity play an important role in pathogenesis of thalassemia. Increased oxidative damage in thalassemia may be due to the depletion of antioxidants such as vita min E and GSH.

p-106
SPLENECTOMY CHANGES THE PATTERN OF CYTOKINE PRODUCTION IN B-THALASSEMIC PATIENTS

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A major cause of morbidity and mortality in β-thalassemic patients is infections, assumed to be the result of immunological changes. To determine the possible defect, we investigated the cytokine production in blood cells of β-thalassemic patients using in vivo and in vitro methods. Heparinized blood samples were collected aseptically from 22 β-thalassemic children aged 10–12 yrs. Half of the patients were splenectomized. Samples from 10 healthy children served as control group. An aliquat of the samples was used for evaluation of plasma IL-2, IL-10 and TGF-β1. Another portion was stimulated with a mixture of LPS and PHA (1 and 10 μg/ml, final concentration), for different time periods (4, 24, 48 and 72 h). Results showed that the circulating TGF-β1 level of splenectomized patients was significantly higher ($p<0.01$) than the control group. In vitro results showed IL-2 production of patients' groups to be significantly ($p<0.01$) lower than the corresponding value obtained for the control group. In addition, IL-10 production by splenectomized group was less than other two group ($p<0.01$), while their TGFβ1 was higher ($p<0.001$) at all time points treated. In conclusion, multi-transfusions could be responsible for a change in the subset of circulating lymphocytes that could contribute to a state of partial immune deficiency in β-thalassemic patients. This is more prominent among splenectomized patients.

p-107
BIOCHEMICAL AND MOLECULAR DIAGNOSIS OF GALACTOSEMIA IN IRANIAN PATIENTS

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Classical galactosemia is an inborn metabolic disorder caused by autosomal recessive mutations in galactose 1 phosphate uridyl transferase (GALT) gene. The incidence of the disease varies from 1:40000-1:60000 among Caucasian population. To date, over 170 different mutations have been reported including point mutations, microdeletions and insertions. The most frequent reported mutation is Q188R which accounts for approximately 60% of mutant alleles in Caucasian population. In the present investigation, results of a 5- year study on galactosemia in Iran including biochemical diagnosis and molecular analysis of GALT gene is presented. Methods: Twenty five galactosemic patients were subjected to diagnosis of galactosemia by the determination of GALT activity in RBCs using Beutler test. DNA samples were investigated for the 5 most reported mutations including Q188R, K285N, X380R, L195P and Q169K using PCR-RFLP method. PCR-SSCP method was used for the whole GALT gene including 11 exons and flanking intronic sequences to investigate the mutations which were not detected by PCR-RFLP method. In a retrospective study, galactosemic patients were traced and their long term outcomes were evaluated. Results: Q188R mutation was the most observed mutation with the allelic frequency of 57.1%. The allelic frequencies for S135L, Y209S, A320T, and K285N were found to be 7.1%, 7.1%, 7.1%, and 3.57%, respectively. Conclusions: Our results show that galactosemia in Iranian population is a heterogeneous disorder at the molecular level. Study on long term outcome of the disease emphasizes the need for a new look and new challenges for galactosemia in Iran.

p-108
DETERMINATION OF THE OPTIMUM GROWTH CONDITIONS OF IRANIAN HALOFERAX ISOLATED FROM URMIA LAKE BY THE TAGUCHI METHOD

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Iranian Haloferax is an extremely halophilic archaeon which exhibits high resistance to ionizing and ultra violet radiation and hydrogen peroxide. It is obtained from the filteration of the urmia lake water, In the recent decade, extremely halophilic archaea have been used in biotechnology. In this study, applying Taguchi method and studying several factors such as PH, temperature, culture medium composition and sodium chloride concentration, optimum growth conditions of Iranian Haloferax were characterized. Considering the Taguchi orthogonal array tables at 5 factors and 4 levels, 16 trials were designed. For each trial, after inoculation, sampling was performed twice (8 AM, 2 PM) and optical density (OD)

was measured by a spectrophotometer at 600 nm. In all of the trials,, the inoculum concentration was 5% and aeration was carried out by shaking at 220 rpm . The dry weight of the cells obtained (g/l) under the above conditions were compared by the Qualitek-4 program . The results showed that temperature was the most effective agent among several factors and there was no direct relation between sodium chloride concentration and culture medium composition. The optimum conditions obtained were as follows: temperature, 47; pH, 7; culture medium B ; sodium percentage , 20% . Therefore the expected growth yield under such conditions will be theoretically 0.0175 grams per liter of bacterial dry weight. In order to validate final results we performed a trial by considering the optimum growth conditions.. The dry bacterial weight was 0.023 grams per liter which is more than the theoretically predicted amount.

Trace Elements

p-109
PROTECTIVE EFFECTS OF SELENIUM AND ZINC ON CHANGES IN CATECHOLAMINE LEVELS OF BRAIN REGIONS IN LEAD INTOXICATED RAT

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The nervous system is the primary target for the lead exposure. In the past few years, increasing considerations have been given to investigate the interaction occurring between toxic metals and some essential metals including Se and Zn with Pb. It has been shown that some trace elements could reverse the toxicity of lead on tissue functions. This study aimed at examining the protective effects of Zn and Se on lead toxicity. Results of short time study showed that intraperitoneal administration of Pb (13.5 mg/Kg) daily for 2 weeks reduced the catecholamine levels of cortex by 25, mid-Brain by 21and cerebellum by 25.6 percent, respectively. Administration of the same amount of lead in combination with either Zn (0.5 mg/Kg) or Se (0.4 mg/Kg) reduced catecholamine levels of cortex by 8.3 and 18.3, mid-brain by 6 and 10.9 and cerebellum 23 and 6 percent, respectively. Daily administration of lead alone (4 mg/Kg) for 60 days reduced catecholamine level of cortex by 27.4 and mid-brain by 47.8 and cerebellum by 39 percent, respectively. When the same amount of Pb in combination with Zn (0.5 mg/kg) or Se (0.4 mg/kg) was administration daily for 60 days, results showed that catecholamine level of cortex was reduced by 9 and 20 and mid-brain by 22.6 and 29 and cerebellum 25 and 16 percent respectively. It is concluded that lead reduced catecholamine levels in different brain regions and Zn or Se might be able to reverse this reduction, and protect brain function to some extent from lead toxicity.

p-110
SERUM ZINC AND SELENIUM LEVELS IN ORAL CONTRACEPTIVE PILL USER

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Oral contraceptive pills (OCP) has been used for several years in Iran. The objective of this study was to investigate the effect of oral contraceptive pill on serum zinc and selenium levels. The results of this study showed that there was no significant difference in serum selenium level in the OCP user compared to the control group (P = 0.935). Relative to the control group, there was significant decrease in the serum zinc level in the OCP user (P=0.009). This study showed a positive correlation between serum zinc level and usage of OCP. As might be expected from the multiple biochemical function of zinc, thus zinc supplementation in OCP user might be of clinical benefit.

p-111
THE EFFECT OF DESFERAL USAGE ON THE LEVEL OF ZINC, COPPER AND FERRITIN IN THE BLOOD OF BETA-THALASSEMIA PATIENTS.

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Iron overload is a constant, and is an important complication in beta-thalassemia patients. They have to use Iron chelating agents such as Desferal to remove iron. Some useful metal ions (Zn^{2+},Cu^{2+}) may be chelated non-specifically. The objective was to assess the effects of Desferal on serum levels of Zn and Cu as well as the effects of dosage and duration of the use of Desferal on the levels of Zn^{2+} , Cu^{2+} and ferritin. Forty beta-thalassemia patients and 40 healthy individual, matched in terms of sex and age, were recruited. There was significant difference between the levels of ferritin of patients (2510.21 ± 26.10 ng/ml) and the control (183.67±4.86 ng/ml) groups. There was also significant difference in the levels of Cu^{2+} of the patients (81.20 ± 3.88 mg/dl) and the control (144.53± 94 mg/dl) groups. However, there was no significant difference in the levels of Zn^{2+} of the patients (88.47± 5.88) and the control (108.36± 6.62 mg/dl) groups. There was a negative but insignificant correlation between higher dosage and longer duration of Desferal use and serum levels of Cu^{2+}. There was also no significant correlation between the dosage and duration usage of Desferal with Zn^{2+} levels. There was a significant inverse correlation between the ferritin level and the dosage of Desferal.

P-112
ALUMINIUM-INDUCED CHANGES IN LIVER CONTENTS OF LIPIDS ARE REVERSED BY COPPER.

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Introduction: Although copper-overload is believed to be hepatotoxic in some diseases such as wilson's disease and childhood cirrhosis, the beneficial effect of this element in lower doses are the subject of widespread investigations. Previously we showed that aluminium could change the metabolism of lipids and lipid-related parameters. This study aimed at examining the protective effect of copper on the liver contents of phospholipids and triglycerides Methods: Male Wistar rats were kept under standard conditions with free

access to food and water. The animals were given acute and chronic doses of aluminium (15 and 1 mg/kg), copper (1.5 and 0.1 mg/kg), and a combination of the two for 15 and 60 days, respectively. Animals were then killed, their livers were removed, homogenized in appropriate buffer, and their contents of phospholipids and triglycerides were measured. Blood samples were also collected, and their sera were separated and used for biochemical analysis. Results: the administration of aluminium changed the plasma lipoprotein levels and lipoprotein lipase activity, which were reversed by copper treatment to some extent. Moreover, alumininum in both acute and chronic doses increased liver phospholipids content form 77 to 97%, but the increase was from 44 to 80% percent in the presence of copper. Liver triglycerides were decreased in aluminium-treated animals by 26 to 33%, but only 11 to 14% decrease was observed in the presence of copper. Conclusion: The present data showed that the toxicity of aluminium on liver functions could be reversed by simultaneous treatment with copper, which might be related to the activation of some copper related enzymes.

p-113
THE CYTOTOXIC EFFECTS OF CADMIUM CHLORIDE (CDCL2) ON THE HUMAN LUNG CARCINOMA (CALU-6) CELL LINE

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Cadmium and Cadmium compounds are known to be harmful to human health, mainly via contaminated drinking water, food supplies, tobacco and industrial pollutants. The aim of this study is to determine the ability of Cadmium Chloride (CdCl2) to cause cell death in the human lung carcinoma cell line (Calu-6). The cell line were grown in RPMI-1640 medium supplemented with 10% FCS, penicillin/streptomycin (100 U/ml, 100 µg/ml) at 37 ° C in 5% CO2/95% air. The cells were plated in 96 multi-well plates. After 24 hours, the medium was replaced with fresh medium containing different concentrations of CdCl2 and incubated for 24, 48 and 72 hours. MTT cell viability test was used to study the cytotoxic effects of Cadmium. Exposure of monolayers to different metal concentrations (1- 1000 µM) in different times showed a significant decrease of viable cells when compared with that of controls. A significant cytotoxicity was observed at 1.0 µM of CdCl2, which reached to the maximum at higher concentrations in a dose-dependent manner. However, the concentrations greater than 200 µM were not associated with further increase in cytotoxicity. We conclude, while high concentrations of Cadmium are harmful to human, lower concentrations induce a significant cytotoxicity in the cancer cells. This finding may introduce a new view on the mode of action and possible application of trace elements in the cancer treatment.

p-114
EFFECT OF NICKEL NITRATE ON SOLUBLE CHROMATIN

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Nickel compounds are well known as human carcinogens, but their molecular mechanism is not fully understood. The objective of the study was to investigate the binding of nickel to soluble chromatin using UV/vis spectroscopy and gel electrophoresis techniques. Nuclei were prepared from rat liver and after brief digestion with micrococcal nuclease. The soluble chromatin was treated with different concentration of nickel nitrate for 45 min at 23°C. The results showed that the absorbance at 260 and 210 nm was decreased as nickel nitrate concentration increased. Turbidity measurement at 400 nm clearly demonstrated the aggregation of chromatin in the presence of nickel. Analysis of the histone proteins content on the SDS gel electrophoresis, revealed disappearance of histone H1 in the presence of nickel, however, the amount of core histone proteins remained unchanged. From the results it is concluded that the effect of nickel on chromatin is dose dependent and results in chromatin aggregation.

p-115
CHANGES IN SODIUM-LITHIUM COUNTERTRANSPORT ACTIVITY FOLLOWING ALUMINIUM TREATMENT

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Sodium-Lithium countertransport (SLC) promotes the exchange of sodium and lithium. There have been many reports confirming the raise in SLC activity in primary hypertension and many other diseases. This study aimed at investigating the effects of Al as a toxic metal on SLC activity using in vivo and in vitro methods. New Zealand white rabbits(n=20) were divided into two groups(n=5 each) for acute and chronic doses .The first group was administered with aluminium chloride intra-peritoneally on alternate days for 2 weeks (25 mg/kg; acute protocol) or for 7 weeks (12.5 mg/kg; chronic protocol). The control groups for each doses received deionized water in similar protocols. Also the effects of different concentrations of Al on SLC activity were studied in vitro. The SLC activity was determined according to the method of Canessa with minor modification. The values of Km and Vmax of SLC were determined using Eadie-Hofstee methods. Acute and chronic doses of AL increase SLC activity, Vmax and Vmax/Km of the transporter but the Km was decreased. Also, in vitro studies showed increased SLC activity and Vmax/Km. So this study suggest that aluminium toxicity (acute and chronic doses) by changing the SLC can induce hypertension.

p-116
SELENIUM AND GLUTATHIONE PEROXIDASE DEFICIENCY IN EPILEPTIC CHILDREN

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Oxidative stress and its resultant free radicals are known to be the both cause and the consequence for epileptic seizures. In order to cease the increasing damage to the brain neurons following epileptic seizures, natural anti-oxidative systems are playing a major role. One of the most important detoxifying systems is composed of the trace element selenium and selenium-dependent detoxifying enzyme namely, glutathione peroxidase. Th present study aimed to examine the serum levels of selenium and glutathione peroxidae in patients with epilepsy. Age-matched 53 epileptic children (29 females and 24 males) and 57 normal subjects were studied during a 15 months period. Blood samples were collected, and their sera were prepared. The sera were used for the determination of selenium level and RBC Glutathione peroxidase activity. There were significant differences in serum selenium levels and RBC glutathione peroxidase activities from the two groups. The findings of the present study strongly support the proposed crucial role for selenium and selenium-dependent enzyme deficiency in the pathogenesis of epilepsy.

p-117

THE EFFFECT OF TITANIUM ON OLEIC ACID TRANSPORT IN RAT EVERTED GUT SACS (EGS)..

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Introduction: Everted intestinal sac technique has been used to estimate the transport of nutrients in rats. A number of factors such as pH and the nature of solvents may play an important role in fatty acid uptake by entrocytes. Previous reports indicate that fatty acid transport is affected by many biochemical parameters including trace metals. Objective: This study aimed at investigating the effects of Ti on oleic acid transport. Method: Male rats (200-250 gr) were killed, their intestines removed, and their jejunum dissected and used to prepare everted gut sacs (EGS). The buffer-filled sacs were incubated in a medium to which oleic acid and $TiCl_3$ had been already added. Then the transported oleic acid inside the EGS was measured spectrophotometrically under different condition of pH, temperature and concentrations. Result: The result indicated that titanium decreased fatty acid uptake by entrocytes in a dose-dependent manner. In the presence of sodium ion at concentrations of 0.5, 1, 1.5, 10 mM, $TiCl_3$ inhibited the uptake of oleic acid by 14.6%, 31.6%, 38.6% and 54.5% , respectively. However, in the absence of sodium ion, the rate of inhibition was 3.5%, 28%, 29% and 39%, respectively. Conclusion: Oleic acid transport appeared to be a sodium-dependent process, and Ti may exert its inhibitory action by interfering with this system.

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THE EFFECTS OF LEAD ON SERUM, LIVER AND BRAIN HIGH AND LOW MOLECULAR WEIGHT ALKALINE PHOSPHATASE IN RAT

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The objective of this study was to investigate the relationship between lead treatment and changes in the activity of serum, liver, and brain high- and low molecular weight alkaline phosphatase. Every other day intraperitoneal injection of 39.5 µmol/kg of lead as Pb (CH3COO)2.3HB2BO), to male rats for 2 consecutive weeks resulted in the decrease of level of liver and brain alkaline phosphatase by 16.7% and 10.9%, respectively. Moreover, an elevation of serum enzyme activity by 28.4% was seen in comparison to untreated controls. Long-term exposure to 13.2 µmol/kg of the lead salt caused statistically significant reduction in liver (18.7%) and brain (13.2%) levels of alkaline phosphatase, and a significant increase (37.6%) in serum activity of the enzyme by 37.6 percent. The serum and liver homogenate from lead treated rats had significantly higher level of high molecular weight alkaline phosphatase, which might be considered as a potential biomarker for lead toxicity.

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LEAD TOXICITY INDUCES LIPID PEROXIDATION AND ALTERS THE ACTIVITIES OF ANTIOXIDANT ENZYMES IN ERYTHROCYTE.

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Introduction: Lead (Pb) has been known as one of the most common heavy metals that causes health problems among chemically-exposed industrial workers like battery industries. It was established that heavy metals like as Pb caused oxidative damage to cells either directly or indirectly by triggering an increased level of production of reactive oxygen species (ROS) that in turn caused damage to the biomolecules such as membrane lipids, proteins, enzymes, nucleic acids. Materials & methods: Blood and morning urine samples from 50 Pb exposed workers and 50 healthy age and sex-matched workers were taken and analyzed twice at an interval of 6 months. Complete blood count (CBC) was done and both Pb-Blood and Pb-urine were measured by graphite furnace atomic absorption spectrometry (Shimadzu, AAS 6800). Pb-Urine values were expressed as observed (Pb-Uob), i.e., without any correction, and after correction for urine specific gravity of 1.016 (Pb-Usg) and urine creatinine (Pb-Ucr), the specific gravity was measured by a refractometer and urine creatinine was determined by a spectrophotometric method. Erythrocyte malondialdehyde (MDA) determined as indicator of lipid peroxidation based on its reaction with thiobarbituric acid (TBA) to form a colored MDA-TBA adduct by used of HPLC. Erythrocyte glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) and catalase activities were determined in washed red blood cells obtained immediately after sampling by spectrophotometric methods. Results & discussion: The results obtained from CBC in Pb exposed workers revealed

mild to moderate anemia with appearance of basophilic stippling in erythrocytes, especially after 6 months. Blood and Urine Lead was significantly higher in case group, and there was significant correlation between blood Pb and urine Pb (r = 0.62) in Pb exposed workers. The level of erythrocyte malondialdehyde concentration, in Pb exposed workers was significantly increased during 6 months (about 30% to 150%) in comparison to healthy ones. There was a positive correlation between the levels of erythrocyte malondialdehyde concentration and blood lead concentration (r=0.73) and Pb-Usg (r=0.65). Erythrocyte catalase activity in Pb-exposed workers was significantly decreased in comparison to healthy ones. There was an inverse relation between the levels of Pb in blood and erythrocytes catalase activity (r= -0.58). Erythrocyte SOD activity increased significantly in case group, and induced in 6 months about 55% to 80% as well as Pb concentration increased, but there wasn't any significant correlation between RBC SOD activity & blood Pb concentration. The activity of erythrocyte glutathione peroxidase increased in low level of blood lead but its activity decline in high blood lead concentration. The results of this study demonstrate severe oxidative stress and failure of antioxidants enzymes to protect cells against to reactive oxygen species, induced by lead toxicity.

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ZINC PLAYS AN IMPORTANT ROLE IN SPERM MEMBRANE LIPIDS PEROXIDATION INHIBITION

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Human seminal plasma includes a variety of trace element such as Zinc that has important role in sperm normal function. Recent study showed that Zinc has antioxidant properties and play an important role in free radical, especially O_2, scavenging. Therefore it is expected that low levels of Zinc has been associated with pathologic effects of free radicals such as sperm lipid peroxidation. Objective: the objective was to study the correlation between Zinc and malondialdehyde (MDA) in seminal plasma of fertile and infertile men .. Methods: Semen samples provided from fertile (n=21) and infertile (n=32) men. The concentration of seminal plasma Zinc and MDA measured by atomic absorption spectroscopy (AAS) and thiobarbituric acid reaction (TBAR) methods, respectively .Results: Concentration of Zinc in seminal plasma of fertile men (13.5± 2.63 mg/100ml) was significantly lower than infertile men (11.62 ± 3.21mg/100ml). Seminal MDA in fertile men (0.74± 0.35 nmol/ml) was significantly higher than infertile men (0.92± 0.27 nmol/ml). There was a negative, but insignificant, correlation between Zinc and MDA concentration. Conclusion: The findings of the study showed that seminal Zinc was approximately associated with lipid peroxidation inhibition, and high level of Zinc was related with low MDA concentration. Based on the findings, it might be possible to suggest the measurement of Zinc in seminal plasma of subfertile men.

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LEAD & DNA INTERACTION

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This study aimed at investigating the interaction of Pb^{2+} with DNA using difference and absorption uv/vis spectroscopy, thermal denaturation, equilibrium dialysis and affinity chromatography. Absorption and difference spectra of the samples prepared by adding various concentrations of lead nitrate into DNA solutions showed that the main changes in the spectra obtained at 215 nm. Lead acetate also gave the same result. Thermal denaturation of the samples showed that at low values of lead, significantl changes in Tm of DNA did not occur, but at high concentrations Tm was reduced to nearly 37 and 45^o C. Equlibrium dialysis was carried out at 6, 24, and 72 h. Because the absorbance of the prepared solutions was increased after 24 and 72h dialysis, only data of the 6 h incubation was used for Scatchard plot analysis. The interaction of lead with DNA on both single and double stranded DNA–cellulose chromatography technique showed a high affinity of Pb^{2+} for DNA, which possibly caused the elution of some DNA molecules from the column.

p-122
INVESTIGATION OF MANGANESE AND IRON ABSORPTION BY RAT EVERTED GUT SAC

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Manganese (Mn) is an essential trace element, and is necessary for catalytic processes and acts as a promoter of various enzymes including the brain-specific glutamine synthesis, superoxide dismutase and pyrurate carboxylase. Although Mn is an essential trace elements for brain function, but manganese toxicity has been reported to cause brain disorder and also disturb heme synthesis. Male Wistar rats, kept under standard conditions, were fasted 24 h prior to the experiments and killed by cervical dislocation. Small intestine was removed, cleaned from debris, washed, blotted dried and weighted. The intestine was cut into small pieces. The segments were everted. The everted gut sacs were filled up with Krrebs- Ringer phosphate (KRP) medium, and suspended in the same buffer medium with or without iron and/or manganese. At different time intervals, the reaction mixture was removed and the concentration of iron and/or manganese inside the sacs was determined. Manganese concentration was determined using flameless-atomic absorption spectrophotometry. Iron absorption by rat EGS was completed within 60 min of incubation, whereas manganese absorption occurred within 30 min. Absorption of both metals by EGS was a dose and time-dependent process. Manganese absorption was reduced by 45% when iron was added to reaction mixture. The absorption of iron was decreased by 15% when manganese was added to the medium.

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COMPARISON OF IN VITRO STUDY OF THE INTESTINAL ABSORPTION OF TITANIUM AND IRON IN RATS

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The objective of this study was to examine the intestinal absorption of titanium and iron and their interaction in rat everted gut sacs (EGS). Incubation of freshly prepared rat EGS in Earls's medium with a pH of 7.4 containing either Ti (IV) and/or Fe (III) showed that showed that the optimum concentration of iron and titanium for intestinal absorption was 300 and 200 µg/dl respectively. Addition of NaF to incubation media caused 13% and 9% decrease in iron and/or titanium absorption. 33% and 24% reduction was seen where ouabain was added to the media. Presence of ascorbic acid in media caused 17% and/or 15% increase in iron and titanium absorption. Iron absorption was reduced approximately 13% when titanium was added to incubation media, whereas addition of iron caused 35% decrease in titanium absorption. The findings suggest that intestinal transport of Titanium as well as iron was saturable, and proceeded through active transport across intestinal mucosal cells.

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THE CORRELATION OF SERUM COPPER AND ZINC CONCENTRATION WITH SOME CARDIOVASCULAR RISK FACTORS

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Introduction: Atherosclerosis is a progressive disease characterized by the accumulation of lipids and fibrous elements in arteries, which is a major cause of morbidity and mortality having multifactoral pathogenesis including both genetic and environmental factors. Other than the high level of LDL-C as a traditional risk factor, serum Copper and Zinc concentration are mentioned as certain risk factors for (CAD) by several prospective and retrospective studies.Objective: the objective of the current study was to evaluate the level of LDL-C and serum Copper and Zinc concentrations. Material and Methods: The study was carried out using sequential sample of 268 (175 males and 93 females) patients who were undergoing routine coronary angiography. Data including Age, smoking habit, menopausal status, history of heart disease, and history of heart disease in family were collected from each patient using a questionnaire. Anthropometric parameters including: weight, height, and blood pressure were measured in all patients. BMI (Body Mass Index) was calculated as weight (kg) divided by height squared (m2).Serum cooper and zinc concentration were measured by Flame Atomic Absorption, while sample lipid profile and blood glucose level were measured by Auto Analyzer RA-1000. Data were assessed by SPSS software. Results: The mean serum Cu concentration in women was more than that in men (P = 0.0003) but mean Zn concentration in men was more than women (P = 0.422). The mean serum Cu concentration in those with the history of heart disease was more than those without (P = 0.065) while the results were in contrast for Zn (P> 0.05). The mean serum Cu and Zn concentration in subjects with the history of high blood pressure were more than those without (P = 0.015, P = 0.548 respectively). The mean serum Cu concentration in those with the history of high blood glucose was more than those without, in contrast of the results of Zn (P> 0.05).The mean serum Cu and Zn concentration in cases with the history of smoking was lower than those without (P> 0.05). The mean serum Cu and Zn concentration in cases having exercise were higher than those without (P> 0.05). The mean serum Cu and Zn concentration in women who were in menopause were lower than those who were not. The mean serum Cu concentration in subjects with BMI below 25 kg/m2 was higher than those with BMI above 25 kg/m2 while the results for Zn were in opposite (P> 0.05). The mean serum Cu and Zn concentration in subject with serum cholesterol above 200mg/dl were higher than those below 200mg/dl (P = 0.0001, P = 0.0024 respectively). The mean serum Cu concentrations in subject with serum TG above 150mg/dl were higher than those below (P> 0.05) while the results for Zn were in opposite (P = 0.001). Conclusion: Some studies revealed that the mean serum copper and zinc concentration in patient with atherosclerosis was higher versus those with a normal angiogram, but there are some other studies reporting a negative relationship between serum cooper and zinc levels and atherosclerosis. The results of the current study demonstrated that a dietary imbalance of Zinc and Copper may be a risk factor in the etiology of coronary heart disease.

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EVALUATION OF ZINC PROTOPORPHYRIN AND URINARY DELTA-AMINOLEVULINIC ACID AS INDICATORS OF LEAD POISONING IN WORKERS EXPOSED TO LEAD

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Introduction: Lead poisoning is a complex disorder affecting many organs in the body, including developing red blood cells, the kidneys, and the nervous system. In lead poisoning zinc is incorporated into protoporphyrin IX instead of iron (Fe^{+3}) and ZPP is produced instead of heme. This metal substitution provides a very sensitive biochemical indicator of lead intoxication. Materials & methods: Blood and morning urine samples from 100 Pb exposed workers (92 men and 8 women) were examined. The subjects worked in storage battery production processes. Complete blood count (CBC)

was done and Blood and urine lead measured by graphite furnace atomic absorption spectrometry (Shimadzu, AA-6800) after wet-ashing of samples. Zinc protoporphyrin was determined by fluorometric method. UALA was determined by colorimetry following ethyl acetoacetate condensation. Serum Iron & TIBC was determined by colorimetric method. As control group 100 normal workers not exposed to Pb were selected. Results & Discussion: The major finding was significant elevated of blood & urine Pb, ZPP and UALA in Pb exposed workers in contrast to normal group ($P<0.001$). The results of this study also showed significant negative correlation between hemoglobin and both zinc protoporphyrin ($r = -0.56$; $P < 0.01$); and blood lead ($r = -0.35$; $P<0.05$). Significantly higher UALA values were found in cases with higher levels of urinary lead ($r = 0.65$; $P<0.001$). There wasn't any statistically significant difference in distribution of serum iron and TIBC levels in the lead-exposed workers and controls. In this lead-exposed population, no correlation between serum iron and blood lead or zinc protoporphyrin was found ($r = 0.02$ and $r = 0.038$). Since blood lead levels (PbB) decrease more rapidly than does ZPP, due to the shorter half-life of PbB (30 days compared to the life span of the RBC which is around 120 days). After workers start being chronically exposured to lead, PbB peaks at 3-6 months where as ZPP peaks later at 6-9 months and stay longer time at peak. This longer half-life theoretically makes ZPP preferable to PbB for evaluating total lead exposure over time. ZPP may be more closely correlated to lead toxicity than PbB. Still, in the setting of industrial exposure to lead, the value of ZPP in detecting subclinical disease, and /or changing occupational medical decisions during surveillance examinations has been emphasized.

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SERUM ZINC AND COPPER LEVELS IN PATIENTS WITH ALZHEIMER DISEASE

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Alzheimer's disease (AD) is a chronically progressive neurodegenerative disease, and is the most common form of dementia. The key protein in the pathophysiology of AD is the amyloid precursor protein (APP), which releases the amyloid-beta peptide (Abeta), an acid peptide molecule involving 39-43 amino acid residues, by proteolytic cleavage. The exact nature of its toxic form is unknown, but could involve a soluble species in equilibrium with the plaques themselves. There is increasing evidence to support that aberrant metal homeostasis is observed in patients with AD. The aberrant hemostasis may contribute to AD pathogenesis by enhancing the formation of reactive oxygen species and toxic abetaoligomers, and facilitating the formation of the hallmark amyloid deposits in AD brains. Also there is a mounting evidence of an unbalanced copper and zinc homeostasis with a causative or diagnostic link to AD. The objective of the present study was to evaluate the relationship between serum copper and zinc levels in patients with AD. Serum copper and zinc levels were measured by atomic absorption spectrophotometry in 60 patients with AD and 60 age and sex-matched healthy controls. Results: A statistically significant negative correlation was found between serum zinc levels and AD. Moreover, there was no significant difference in serum levels of copper from patients and controls.

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Basic Biochemistry

Advanced Techniques in Biochemistry
Biochemistry of Microorganisms and Parasites
Biophysical-Chemistry
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Advanced Techniques in Biochemistry

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EFFECT OF CHLORIDE ION ON THE CIRCULAR DICHROISM SPECTRA AND DETERMINATION OF SECONDARY STRUCTURE OF THE PROTEIN

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Circular dichroism (CD) is being increasingly recognized as a valuable technique for examining the structure of proteins in solution. However, the value of many studies using CD is compromised either by inappropriate experimental design or by lack of attention to key aspects of instrument calibration or sample characterization. The objective of the study was to examine the effects of chloride ion on the CD spectra using an optimized solvent/buffer system. Mouse monoclonal antibody were purified and dissolved in 15 mM phosphate buffered saline (PBS) at pH 7.2 with a chloride concentration of about 0.13 M. a CD spectrum of antibody was recorded in far-UV region (190-250 nm) by JASCO spectropolarimeter model J-810 and then a secondary structural element of protein was estimated. The HT voltage increased below 205 nm, leading to drastic noisy effect on the CD spectra. Analyses of CD spectra for estimation of protein secondary structure revealed high RMS and unusual secondary structural elements. Antibody classified as all-β protein, but in this solution low content of β-sheet observed. By replacing the chloride ion by fluoride ion the CD spectra of antibody improved, RMS decreased in reliable range and β-sheet content of antibody became significant. Thus absorption of chloride ion about 200 nm increased RMS and may affect the accurate secondary structure estimation. In contrast, fluoride ion does not absorb significantly in this spectral range and is the best alternative for maintenance of ionic strength in the CD Studies.

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DEVELOPMENT OF AN ELISA BASED ON RECOMBINANT VP2 PROTEIN EXPRESSED IN E.COLI FOR THE SEROLOGICAL DIAGNOSIS OF INFECTIOUS BURSAL DISEASE VIRUS

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Infectious bursal disease (IBD), also called Gumboro disease, is a highly contagious viral disease. It causes heavy economic losses to the poultry industry worldwide, either by causing a high-mortality acute condition or by leading to immuno-suppression in young chickens provoked by the destruction of immature B lymphocytes within the bursa of Fabricius. It is caused by IBD virus (IBDV), which belongs to the genus Avibirnavirus of the family Birnaviridae. The IBDV genome consists of two segments of double-stranded RNA designated as A and B. Segment A encodes a 108-kDa polyprotein that is self-cleaved to produce VPX (48 kDa), VP3 (32 kDa), and VP4 (28 kDa). In the mature virions, VPX is processed into VP2 (41 kDa). VP2 and VP3 are the major structural proteins of the IBDV virion. VP2 has been identified as the main host-protective antigen of IBDV and carries major neutralizing epitopes. The routine technique for detecting antibodies specific to IBDV is serological evaluation by enzyme-linke immunosorbent assay (ELISA) with preparation of whole virus as the antigen. The aim of the study was to develop a new antigen through the expression of the VP2 in E coli and use it directly, purification directly. The antigenecity of the protein was similar to that of the native virus. The VP2 antigen was tested in a new capturing ELISA with more than hundred chicken sera. There was an excellent correlation between the results of the ELISA using unpurified VP2 and those of the commercial kit. The findings suggest that the VP2-based ELISA is a good alternative to conventional ELISAs, which use whole virions or proteins expressed in eukaryotic.

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PRELIMINARY SCREENING OF DIFFERENTIAL PROTEOMICS ANALYSIS WITH CARRIER AMPHOLYTE ISOELECTRIC FOCUSING BASED TWO DIMENSIONAL ELECTROPHORESIS

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Two dimensional electrophoresis (2-DE) is a commonly utilized method for separating complex protein mixtures and preparing the map of protein expression profile of a given sample in different states. It can separate hundreds to thousands proteins in one single experiment according to isoelectric points and molecular mass of the proteins. This method is practically carried out by two acryl amide based gel electrophoresis, namely isoelectric focusing (IEF) in the first dimension and SDS-PAGE in the second dimension. Two techniques are applied to IEF process on a gel matrix for 2-DE. One of them is based on carrier ampholytes (CA) to create pH gradient in a tube gel system and the other uses immobilized pH gradient (IPG) strips to make stabilized pH gradient. The IPG-IEF has several advantages over CA-IEF, which make it the first option to do 2-DE. In this study, after making some improvements in CA-IEF method, in order to determine the screening capability of analytical scaled systems, we examined the current and improved CA-IEF methods and also the IPG-IEF method, in a given proteomics model. Remarkably, despite of obtaining the higher resolution power in 2-DE gels prepared by 7 cm IPG-IEF, the final picture of differentially expressed proteins by the improved CA-IEF method and the IPG-IEF were not deeply changed. Also in comparison with current carrier ampholyte isoelectric focusing method, this method showed higher resolution power to appear major expressional changes in proteomic samples and demonstrated can be used in order to show the main expressional modifications and a whole image of sample preparation efficacy as a nice substitution for immobiline based IEF method.

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PRODUCTION OF ANTIBODY USING PROTEIN IN GEL BAND: RAISING ANTIBODY AGAINST THE 26-KILODALTON PROTEIN ANTIGEN OF HELICOBACTER PYLORI

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Introduction: Helicobacter pylori (H. pylori) colonize the stomach of half of the world s population, causing a wide spectrum of diseases ranging from asymptomatic gastritis to ulcers to gastric cancer. During the past several years stool antigen tests for detection of H. pylori infection has drawn a great deal of attention among clinical laboratories.
Objective: The objective of the present study was to establish high specific polyclonal antibody against the 26 kDa protein antigen of H. pylori that could be used to produce a diagnostic stool antigen test. Materials and Methods: Preparative sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of whole cell protein extract from H. pylori was performed according to Laemmli method and stained with Coomassie Brilliant Blue R-250 (CBB R-250). The part of gel containing the 26 kDa protein band was excised and removing CBB-R250 from the part of gel was carried out according to Ball method. Polyclonal antibody was raised in adult New Zealand white rabbits by intramuscular injection and several subcutaneous injections on the back of rabbits with homogenized protein band and Freund adjuvant. After the second and the third injections, the rabbits were bled and their sera were tested against whole cell protein extract and the purified electroeluted 26 kDa protein antigen .Results: The antibody titer as a measure of quality 1:1000 dilution in an indirect enzyme immunoassay system was determined. The specificity of the antiserum was further identified by immunoblotting system in which the antiserum reacted with the purified 26 kDa protein antigen and whole cell protein extract from H. pylori in addition intact cells of H. pylori.

Conclusion: This method is rapid and gives high specific polyclonal antibody without any purification of serum or antigen and the antibody would be useful in stool antigen test for detection of H.pylori infection.

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ELECTROELUTION AS A SIMPLE AND FAST PROTEIN PURIFICATION METHOD: PURIFICATION OF THE 26- KILODALTON PROTEIN FROM HELICOBACTER PYLORI

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Introduction: Helicobacter pylori (H. pylori) causes gastric infection in more than half of the human population worldwide and >80% of population from developing countries. The analysis of H. pylori proteins is necessary for the elucidation of virulence factors, antigens and vaccines, all important for diagnosis, therapy and protection. In the pre-genomic era the purification of proteins was mostly performed using detergent treatment of the bacterial cells, ultra-centrifugation, various chromatographic methods, antibody detection, N-terminal sequence determination and finally cloning and identification of the corresponding gene. Objective: The objective of the study was to describe a rapid and reliable method for protein purification from H. pylori.Materials and Methods: For whole cell protein extraction, the bacterial cells were ruptured by octly-β-D-glucopyranoside. The isolation and purification of the 26 kDa protein were attempted by various techniques including ammonium sulfate precipitation, dialysis, preparative sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and electroelution .Results: Assuming that the total protein content of cells is 30% on a dry weight basis, then the expected maximum protein content in 0.92 g (dry weight) of bacterial cell lysate in a volume of 25 ml PBS, pH 7.4 would be 11 mg/ml, assuming 100% protein release. In practice, the total intracellular protein 3.5 mg/ml was determined by completely extracting the protein from the cells with 4 M sodium hydroxide. The details of protein purification are given in following protein purification table .
Purification step Volume (ml) Protein(mg/ml) Total protein(mg) Recovery(%Total protein)
Crude homogenate 50.0 3.0 150 100
35000xg Supernatant 43.0 2.0 94 63
Ammonium sulfate fraction 5.0 14.0 70 74
After dialysis 6.0 8.9 45 63
Electroelution 3.0 0.3 0.89 2
Conclusion: The method is a relatively short and efficient procedure and yields pure the 26 kDa protein preparation free of contaminating proteins.

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SOLID-PHASE FLUOROIMMUNOASSAY OF SERUM HTSH USING ANTIGEN-FITC

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Different fluoroimmunoassay methods have been used for thyrotropin (hTSH) measurement. The objective of the study was to examine a solid-phase fluoroimmunoassay with FITC labeled antigen (F-TSH) to measure hTSH. The hTSH monoclonal antibodies, immobilized on polystyrene tube, were used for competitive binding of serum sample hTSH and F-TSH. The fluorescein-labeled TSH (F-TSH) were prepared by overnight incubation of fluorescein isothiocyanate (FITC) and commercial pure TSH, followed by gel filtration (G-25) purification to obtain pure F-TSH. The constructed standard curve of F-TSH was linear in range of 0.015-15 mIU/L. The inter-assay coefficient of variation (CV) were 7.3% , 11.2% , 6.3% at 0.015 , 0.5 and 15 mIU/L TSH, and intra-assay CV were 10.2% , 6.0% and 7.7% at the same TSH concentrations, respectively. A good correlation was found with radioimmunoassay ($r=0.91$, $P<0.005$). The findings of the study show that the assay method has suitable sensitivity that allows the identification of primary hyperthyroidism. The assay is fast and technically simple, and inexpensive, which is ideal to use in routine and normal clinical laboratories.

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PROTEOMIC CHARACTERIZATION OF PROTEINS EXPORTED BY THE PHYTOPATHOGENIC BACTERIUM ERWINIA CHRYSANTHEMI

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Erwinia chrysanthemi is responsible for the soft-rot disease in a wide range of plants of agricultural importance. Pathogenicity of this bacterium depends on the production and secretion of degrading enzymes such as pectinases, cellulases and proteases. The objective was to study the extracellular proteins of E. chrysanthemi using a proteomic approach. Extracellular proteins were isolated from E. chrysanthemi culture supernatants in the presence or absence of inducers from plant origin. By analysis of mutant, Western blotting and mass spectrometry (MALDI-TOF) 55 spots representing 25 unique proteins were identified. While proteases and a cellulase are constitutively produced, about fifteen pectinases are induced. Moreover, we identified another secreted protein, AvrL, homologous to an avirulent protein of Xanthomonas campestris and demonstrated that its export necessitates the Out system involved in pectinase secretion. A complementary analysis of the E. chrysanthemi periplasm was performed. In this compartment, we found mostly proteins involved in active transport or substrates either identified, or predicted by sequence homologies, or totally unknown. The inducible proteins are mainly involved in pectin catabolism or in iron assimilation, two essential factors of E. chrysanthemi virulence.

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MEASUREMENT OF PLASMA MDA USING HPLC METHOD WITH FLUORESCENCE DETECTOR

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Malondialdehyde MDA is a tree-carbon aldehyde which is a marker of lipids peroxydation. It is measured as a part of thiobarbituric acid reactive substances (TBARS). Very low amounts of TBA2-MDA complex can be potentially detected and measured by HPLC method with fluorescence detector. This study was carried out to measure plasma MDA using HPLC method with fluorescence detector. Blood samples were taken from forty-eight healthy young women, and then plasma samples were prepared. The most common method for measurement MDA is based on its reaction with 2-thiobarbituric acid (2-TBA) in acidic media at 100 ºC and measuring absorbance at 525 nm (excitation) and 560 nm (emission). Separation was performed with a 4 mm (i.d.) × 125 mm chromatographic packed column with Eurosphere 100 C18 (5µm particle diameter) and mobile phase: 40/60 (by vol) methanol-KH2PO4 (pH: 6.8) with 0.8 mL/min flow rate. Mean plasma MDA concentration was 1.29 ± 0.25µM. Correlation between concentration and peak area was completely linear (R2: 0.997). Recovery was 90-93%. The within and between precisions were 3% and 7%, respectively. Detection limit and retention time were 0.005 µM and 4±0.1 min, respectively. In conclusion, the introduced HPLC method with fluorescence detector has high accuracy and precision for detection and measurement of TBA2-MDA complex.

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QUANTITATIVE HPLC ANALYSIS OF MONOTERPENALDEHYDES IN DIFFERENT SOURCES OF IRANIAN SAFFRON

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Saffron is the dried stigmas of a flower scientifically identified as Crocus sativus L. Although the source of saffron is unknown, it apparently originated in the area of Iran and Greece. While the world's total annual saffron production is estimated to be 190 tons, Iran produces about 80 of the total. This study was designed to analyze the monoterpene aldehydes of Iranian Saffron. Four certified saffron samples (Crocus sativus L.), one each from different types of Iranian saffron: Ehteshamiyeh, Tarvand, Sabagh, Abbaszadeh were analyzed using an HPLC UV detection method. This analysis quantified the 2 major monoterpene aldehydes of saffron in each sample and their concentration was analyzed at two different wavelengths. Ehteshamiyeh and Tarvand saffron extracts possessed the highest concentrations of water-soluble picrocrocin and Tarvand and Sabagh saffron extracts possessed the highest concentrations of safranal. The results indicate that the differences might be due to the origin of the sample, to the dissimilar drying processes possibly involving varied time periods, as well as to storage conditions. Finally, the simple and specific HPLC method developed in this study can be used for the quality control that allows for quantification of the major biologically active monoterpene aldehydes in different saffron samples from Iran.

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STUDY OF CARTILAGE MATRIX AFTER AUTOLOGOUS GRAFTS TO DIFFERENT MATRIX BY HISTOCHEMICAL TECHNIQUES

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The extracellular matrix (ECM) plays a significant role in the regulation of tissue growth, development, and homeostasis. Molecules in the extracellular matrix can sequester and release growth factors during tissue remodeling as was recently shown with vascular endothelial growth factor in hypertrophic cartilage. Cartilage is a specialized form of connective tissue containing chondrocytes which are surrounded by an extensive intercellular matrix. Cartilage consists of matrix glycoproteins, the aggregating proteoglycans and collagens. These proteins have also been proved to be effective in growth factors, and are important in the concerted regulation of cellular growth and differentiation by growth factors and extracellular matrix. Although chondrocytes are able to produce matrix components throughout life, their production can not keep pace with the repair requirements after acute damage to hyaline or articular cartilage. The extracellular matrix of these "repair tissues" is poorly integrated with the matrix of the damaged cartilage. Cartilage is rather well suited for transplantation due to slow metabolism of the chondrocytes and low antigenic power of cartilage. However, it is difficult but not impossible for antibodies or cells of the immune system to diffuse through the matrix into the cartilage. The aim of this study was to compare the different matrices such as muscular epimisium, stromal, gingival and dermal skin on cartilage development. Fifteen New Zealand white rabbits (2.5-3 kg) were used. Four millimeter holes were made in rabbits' pinna using punching techniques. The Rabbit's pinnas cartilage tissues were then placed in different matrices. Characteristic of the matrix tissues were evaluated by conventional histochemical methods after10, 20, and 30 days. Toluidine blue made significant contrast between density of glycosaminoglycans in different matrices. Histochemical observations demonstrated that chondrocytes in different matrices can affect glycoprotein synthetic procedures.

p-137
PURIFICATION OF TWO ANTIGENS EXTRACTED FROM BORDETELLA PERTUSSIS BY AFFINITY AND ADSORBTION CHROMATOGRAPHY

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Whooppig cough (Pertussis) is a severe, highly contagious respiratory disease especially in young infants. Pertussis whole cell vaccine has been effective but at the same time has been the subject of considerable debate with regards to safety. Pertussis vaccine is mainly composed of two extracellular proteins including pertussis toxin (PT) and filamentous hemagglutinin (FHA). These have been considered of two potential candidates for production of acellular vaccine. The aim of this study was to purify these two antigens from submerged cultures of B. pertussis using affinity and absorption chromatography. For this purpose B. pertussis strain RIVM - 134 was grown in a 45-litre fermentor using a modified Stainer–Scholte medium supplemented with dimethyl (2, 6-o-) β -cyclodextrin . The two antigens (PT and FHA) were extracted from the supernatant of the culture by centrifugation .The suspension was then concentrated and submitted to chromatography for purification of the above antigens . Pertussis toxin was obtained from one of the fractions in Fetuin-Sepharos 4B column and FHA from a certain fractions in hydroxylapetite column .They were dialyzed against PBS and protein concentration were estimated to be 1200, and 950 µg/ml, respectively . The identities of both antigens were confirmed by SDS- PAGE and immunoblotting techniques. The results showed that the procedures are highly practical and can be standardized for large scale preparation of PT and FHA for the production of an acellular vaccine.

p-138
HPLC ANALYSIS OF TEAR PROTEINS IN PATIENTS WITH RHEUMATOID ARTHRITIS

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The tear film is composed of three layers namely, lipid, aqueous and mucin. It has many functions including protecting the ocular surface. The tear film covering the ocular surface presents a mechanical and antimicrobial barrier and ensures an optical refractive surface. The most important part of the tear system is its aqueous layer that forms the biochemistry of the tear film. The aqueous component contains electrolytes, water, and a large variety of proteins, peptides and glycoproteins, and is primarily secreted by the lachrymal gland. Quantitatively and qualitatively, its composition must be maintained within the fairly narrow limits to maintain a healthy and functional visual system. Rheumatoid arthritis (RA) is an autoimmune disease that causes chronic inflammation of the joints. The disease can also cause inflammation of the tissues around some joints, as well as other tissues within human body. As activated neutrophils play a crucial role in the destruction of synovial joints in rheumatoid arthritis, in several pathological instances, the lachrymal gland can become a target of the immune system and show signs of inflammation. This can occur as a result of autoimmune diseases. This study aimed at analyzing tear proteins using analytical methods including SDS-PAGE electrophoresis, spectroscopic protein assay and HPLC. Twenty five female volunteers (40-60 years old) were given a written consent including various questions regarding their general health and the history of the disease. Stimulated tear samples were collected using sterile capillaries, marked and stored frozen for later use. Poly acrylamide gel electrophoresis (PAGE) and high performance liquid chromatography (HPLC) were the techniques of choice used throughout the study. The results showed a marked decrease in the biological activity of lysozyme and a different electropherogram for R.A sufferers compared to healthy cases.

p-139
TOTAL TEAR PROTEIN CONCENTRATION DECREASES DURING RAMADAN FASTING

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The tear film is composed of three layers named lipid, aqueous and mucin. Human tear has many functions the most important of which is protection of the ocular surface. The tear film covering the ocular surface presents a mechanical and antimicrobial barrier, and ensures an optical refractive surface. The aqueous component of it contains electrolytes, water, and a large variety of proteins, peptides and glycoproteins, and is primarily secreted by the lacrimal gland. The composition of tear film should be remained in a narrow range both quantitatively and qualitatively in order to maintain a healthy and functional visual system. Muslims abstain from food and drink from sunrise down to sunset during the holy month of Ramadan. It is expected that an extended strict fasting may influence tear secretion and quantity. In this research, we compared the levels of tear proteins during Ramadan fasting period and compared with protein content of the volunteers during other months of the year when they were on a normal diet. The analytical methods used in this study included electrophoresis, spectrophotometric techniques and HPLC analysis. Tears of thirty volunteers (20 males and 10 females, 22-27 years old) were stimulated using onion vapor for 30 seconds. Samples were collected using sterile capillaries, marked and stored frozen until use. The results showed that the total protein concentration (TPC) and some of other tear components such as lysozyme had decreased in samples collected during fasting. Besides, comparing the electrophoretic tear protein pattern in fasting and non- fasting states also showed some alterations.

p-140
COMPARISON OF THREE DIFFERENT EXTRACTION METHODS FOR CELL MEMBRANE PROTEOME ANALYSIS

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The separation of membrane proteins is a challenging procedure due to their high hydrophobic properties. This study aimed at examining three different commercial membrane protein extraction kits to determine the most effective one for further protein analysis. The Monocyte/Macrophage cell line J774.1A was cultured in RPMI medium supplemented with 10% FCS and 100IU/ml penicillin and 100mg/ml streptomycin. Complete formation of monolayer cell on 75cm^2 cell culture flasks which included 4-5x10^6 cells were treated for protein extraction. Ready prep sequential extraction kit

(Bio-Rad), Mem-PERR Eukaryotic Membrane Protein Extraction Reagent Kit (PIERCE), and Proteo Extract TM Native Membrane Protein Extraction Kit (Merck) were used separately for membrane protein extraction. Concentration of membrane and cytosolic protein fractions were determined with Bradford assay and confirmed on SDS-PAGE gel. The proteins obtained from each phase were used in 2-D gel electrophoresis with IEF in first dimension and SDS-PAGE in second dimension. In addition, each sample was used in a 16BAC/SDS-PAGE with high capacity in separation of hydrophobic proteins. 16 BAC is a detergent which confers positive charges to hydrophobic membrane proteins and avoids hydrophobic protein aggregation during SDS-PAGE. The results indicated that each method has different extraction capacity for membrane proteins and protein profiles obtained from each membrane fractions showed different patterns. Therefore, the choice of the extraction method could only be done empirically on the basis of the goal of designed experiments.

p-141
A MODIFIED METHOD FOR PURIFICATION OF BASIC PROTEIN (NUCLEOPROTEIN) OF INFLUENZA A VIRUS

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The influenza virus annually causes substantial human loss due to its antigenic changes. Influenza vaccines are prepared upon identification of virus subspecies that emerge every year. Since the changes in the glycoproteins expressed on virus surface influence its immunogenicity, vaccines produced so far have been unable to induce long term immune response. Numerous studies have shown that the sequence of the protein attached to RNA, nucleoprotein, remains unchanged in various strains. Therefore, this protein could be considered as a potential vaccine candidate with a long-lasting effect. The protein is present in low quantities and so its purification from virus culture is challenging. In a majority of reports sucrose gradient has been applied for this purpose. The objective of this study was to adopt a simple procedure using detergents and high-speed centrifugation to extract protein. The extract was concentrated using microcone. The results of these experiments showed that the intensity of protein bands on SDS-PAGE have been multiplied compared to reports available in the literature.

p-142
AN AFFINITY CHROMATOGRAPHIC PURIFICATION OF THE PORPHYRIN-SPECIFIC MITOCHONDRIAL MEMBRANE RECEPTOR USING A NEW TYPE OF SYNTHETIC LIGAND

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The objective of the study was to examine a reliable and one-step-simple technique to isolate and purify a porphyrin IX(k)-signaling receptor from the rat heart muscle mitochondrial membrane total protein mixture. A key stone of the method is an application of a novel Agarose-6B-CL-linked affinity ligand, C17 (epoxy)-2-cyclohexyl-fullerence(C60)-2-(butadiene-1-yl)-tetra(o-γ-aminobutyryl-o-phthalyl)-porphyrin-k, which is found to be the most efficient molecular trap used in a reversible recognition of porphyrin-binding proteins. To prepare a chromatography suitable sample, a scalar 40-70% ammonium sulfate saturation treatment has been employed first over the above mentioned protein mixture with a following dialysis against a column equilibrating buffer (10 mM potassium phosphate (pH 5.45)/15 mM EDTA) and a subsequent fast lyophilization. A column size used was 1.4×16 cm. Once 1.5-2.0 ml sample (150-200 μg protein per 1.0 ml equilibrating buffer) applied onto the column, all zero affinity compounds were to remove with equilibrating buffer and then a combined linear gradient elution (10 mM-18 mM KH2PO4)pH 5.45)/5.0 mM-7.5 mM NaCl (pH 5.45)) has been started, 0.7 ml/min, 22 ºC, total gradient volume 200 ml. The porphyrin high affinity (k=1.75, 8.6×104 μM/ml) peak was clearly separated at Ve =168-170 ml. According to 10%-PAAG SDS (0.25%) and SDS-free electrophoresis versions, the receptor isolated is ~17.0 kDa protein monomer, perfectly homogenous with no sign of other protein/peptide contaminants. This is a predominantly α-globular protein, as seen from its CD-spectra, with a relatively high content of hydrophobic and heterocyclic residues revealed by UV-and Near-IR spectroscopy tests. The method proposed, may serve as a convenient tool in research on porphyrins and/or fullerene-porphyrin.

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p-143
A LONG-TERM SURVEY ON THE PREVALENCE OF EHEC ESSENTIAL VIRULENCE GENES IN ESCHERICHIA COLI STRAINS ISOLATED FROM HUMAN AND ANIMAL SPECIMENS

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Enterohemorrhagic Escherichia coli (EHEC) are important bacteria due to the ability to produce Shiga toxins (STX). Shiga toxins and other numerous potentially virulence factors such as enterohemolysins give them the ability to cause gastroenteritis such as diarrhea, dysentery, and hemorrhagic colitis (HC), and also life-threatening sequels such as hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP). 180 Escherichia coli strains isolated from more than 350 animal (cattle, sheep, dog, and cat) stool and milk samples, and human feces collected in Tehran between 2003 and 2006 were examined by PCR in order to detect stx1, stx2, and ehxA genes. In this method, three primer pairs were used to identify highlighted genes. Of a total number of 180 Escherichia coli isolates, 19 strains included Shiga toxin-encoding genes (3 stx1+ and 16 stx2+ strains), and 12 strains contained enterohemolysin-encoding genes (ehxA). By comparison, the results are relatively in concert with other studies. This means that the prevalence rate

of Shiga toxin-encoding genes (stx1 and variants of stx2) was regular for animal samples but was beyond expectation for the human specimens (max 1%). stx2 gene prevalence was more than stx1 gene prevalence, as expected. On the other hand, the number of enterohemolysin genes (ehxA) found, shows a significant decrease contrary to other studies. Finally, the difference between the data could be due to differences among the distribution of phages carrying genes, environmental conditions (food, drinking water, stress etc), climate (temperature, humidity etc) or age and sex of hosts.

p-144
AN ATTEMPT TO DETECT ESCHERICHIA COLI VEROTOXIN- AND ENTEROHEMOLYSIN-ENCODING GENES IN FECES BY MOLECULAR BIOLOGY METHODS

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Since their initial recognition in more than 25 years ago, Verotoxin-producing Escherichia coli strains (STEC) have emerged as important causative agents of human and animal diseases and their life-threatening sequels. Direct detection of Escherichia coli ehxA, stx1, and stx2 virulence genes in human and animal feces was done by PCR and RAPD as rapid and specific assays due to the important health and economic aspects of these strains. We used multiplex PCR and RAPD assays for detection and genetic characterization of highlighted genes in 184 Escherichia coli strains (105 from humans and 79 from cattle) isolated in University of Tehran Faculty of Veterinary Medicine. In PCR and RAPD studies of a total of 184 human and animal stool samples, we found no highlighted genes. As expected, direct detection of genes was difficult in feces. Contrary to the use of several DNA extraction methods (e.g. boiling, bacterial enrichment, inhibitor deletion, use of DNA extraction kit etc) and several attempts, final results revealed no success. Reasons for this situation include the presence of strong chemical inhibitors such as bile acids, salts, and pigments and different cyclic and phenolic substances within stool that interfere with PCR. However, to extract DNA from feces, we need to use more sensitive assays such as column chromatography or magnetic glass beads that are too expensive and time-consuming , but will fulfill our research objectives.

p-145
INVESTIGATION OF GAG GENE OF BLV IN WHITE BLOOD CELLS OF INFECTED COWS

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Bovine leukemia virus (BLV) from delta retrovirus genus and retrovirus family has three structural genes named gag, pol, env. In this study, 100 whole blood and serum samples obtained from affected cattle over two years from some regions in Iran were examined for gag gene investigation in two stages. In the first stage, the samples were examined by indirect ELISA. Thirty three cases (33%) had antibody against gp51 antigen from BLV. In the second stage, 20 positive serum samples and 15 serum samples from apparently healthy cattle were examined by conventional PCR method for gag gene. In this stage, all of the positive serum samples and 3 of the negative samples showed specific 1184 bp segment for gag gene.

p-146
RNA AND GLYCOGEN CONTENT IN TISSUES OF TRICHINELLA

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The maintenance, cyto - and histo-topographies of RNA and glycogen are one of the criteria of morphofunctional conditions of tissues, in particular intensity of synthesis of fibers and tissue carbohydrate exchange. The purpose of this research was to study dynamics of RNA and glycogen in tissues of Trichinella larvae and in the host (rat) muscles infected with Trichinella spiralis and T. pseudospiralis, both in intact parasitic systems, and after the action of phytohaemagglutinin (PHA). PHA was given with forage in a dose of 70 mg/kg body weight. Muscle tissue samples were collected from animals under light anesthesia on the 35th day of infection. RNA was detected by Brachet reaction and polysaccharides by the method of Schabadasch with the application of the Schiff-reagent. It was shown, that the destroyed muscular tissues of the infected rats had negative reactions for RNA and glycogen.. In an external layer of the capsules surrounding T.spiralis larvae, positive reaction for the presence of polysaccharides was noted, whereas glycogen and RNA were absent from intracapsular sarcoplasm. After application of PHA for 5 days before infection of the rats, a slight rise in RNA and glycogen of muscle fibers was noted compared to the infected control group. In tissues of both species of Ttrichinella larvae, RNA and glycogen maintenance and distribution were close to those of the muscles of the larvae of Trichinella infected control rats.

p-147
TWO DIMENTIONAL MAP OF SHIGELLA PROTEOME

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Shigella spp. is among the most important etiological factors for people who are living in developing countries and travelers to tropical countries. Shigellosis is a major public health problem in infants and young children in developing countries and is the major etiologic agent of traveler's diarrhea. The bacteria are spread via the faecal–oral route. Once ingested, the virulent organisms invade the colonic epithelial cells, multiply intra-cellularly and spread to adjacent uninfected intestinal cells. Invasion of host cells by Shigella requires expression of virulence genes located on a 230 kb plasmid. The products of these genes include the Ipa invasins, the Mxi and Spa proteins, and the IcsA, IcsB and VirA proteins. The first protein mediates the invasion of gut epithelia and macrophage apoptosis. The second two form a type III secretion system. And the last three are responsible for intercellular spreading of bacteria in the lower gut. Increasing

recognition of the burden of the incidence and mortality rates of infections caused by Shigella spp. has stimulated research to develop vaccines to prevent shigellosis. Two approaches for Shigella vaccine development are applied including development of attenuated strains without a heterogeneous gene and attenuated hybrid bivalent vaccine strains with a heterogeneous gene which can code to express highly protective antigens. Accumulating data indicated that the major protective antigen appeared to be the type specific O-antigen because the presence of antibodies against the serotype-determining lipopolysaccharide (LPS) O-antigen was correlated with protection against disease. However, recent studies have shown that more protective antigens, which can screen from immunogenic proteins, may be selected as vaccine candidates. Recently, proteomics, a high-efficiency technology with high accuracy, has brought new challenges into this area and it provides an understanding of the expression of a genotype at the phenotypic level in a target cell at a given stage. Much of information about immunogen amounts or activities can be derived from proteomics with Western blotting, namely immunoproteomics. Research on vaccine technology will be improved by quick identification of proteins in the science of proteomics. In this study, extracellular proteins from the culture medium and whole cell proteins in cellular extracts of S. dysenteriae were examined by two-dimensional (2-D) gel electrophoresis using immobilized pH gradient (IPG) technology. After staining, gels were analyzed by Melanie software. The results indicate that most of the protein spots underlie between pH 4-7 and molecular weights of 1600 -200000 daltons.

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DECREASED ACTIVITY OF GLUTATHIONE PEROXIDASE IN ANKYLOSING SPONDYLITIS

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Backgorund: Ankylosing spondylitis (AS) is a distinct disease entity characterized by inflammation of multiple articular and para-articular structures. Studies have demonstrated the role of oxidative stress in pathogenesis of Rheumatoid arthritis. Objective: To evaluate the association of oxidative stress and AS, we measured the activity of glutathione peroxidase (GPX) in the affected patients ancompared it with a control group. Material and Methods: Erythrocyte GPX activity was evaluated in 87 documented AS patients and was compared with 93 apparently healthy control people. Results: GPX was 5153 ± 555 and 10998 ± 288 SEM IU/g Hb in the AS and the control group, respectively. Discussion: GPX activity was significantly lower in the AS group ($P<0.001$). The lower level of GPX in AS group may be due to the chronic nature of this inflammatory disease and it can be regarded as an indicator of antioxidant response.

p-149
DETECTION OF BACTERIAL MARKERS IN THE BLOOD OF PATIENTS WITH FAMILIAL MEDITERRANEAN FEVER

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The molecular mechanisms of microflora and the host interaction in the development of familial Mediterranean fever (FMF) were studied. The bacterial markers (metabolites, which are produced by the host automicroflora) were identified by gas chromatography-mass spectrometry (GC-MS) method. This method allows the simultaneous screening of a large number of bacterial markers in one sample of clinical material. The blood samples of healthy controls (n=48) and FMF patients in attack (n=29) and remission (n=65) periods were analyzed. The results indicate the cardinally biased and unusual profile of bacterial markers for all investigated groups. Notably, none of the bacterial markers specific for FMF was detected. Discriminant analysis (DA) revealed that different stages of FMF were distinctly characterized by the set of markers of bacterial origin. The results of DA of pairs of studied groups indicate that there is no complete coincidence of significant markers for discrimination of different pairs of groups, which is reflecting multiplicity of bacterial agents involved in disease activity. The changes in correlation of bacteria in FMF were revealed with the emergence of a new functional relation not proper for norm; an increase of bacterial load and a positive correlation in the stage of remission were observed unlike that of the attack. It seems, that patients with FMF – characterized by genetically determined excess reactivity – realize an inflammatory potential through attack when the level of bacterial load in remission period amounts to critical, i.e. triggered launching of an inflammatory response of the organism occurs.

p-150
A STUDY OF INTESTINAL ALKALINE PHOSPHATASE ISOZYMES IN THE SERUM OF ADULT GIARDIASIS PAITENTS

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Abstract Human giardiasis is an infection produced by flagellate protozoan, Giardia lamblia that is localized to the small bowel where it produces damage to the mucosa with or without gastrointestinal symptoms. A number of reports have described some changes in intestinal alkaline phosphatase in rat infected with giardiasis . Furthermore, we observed elevated levels of serum intestinal alkaline phosphatase (IALP) in humans infected with giardiasis by an electrophoretic method. We evaluated a group of 67 patients infected with giardiasis attending diagnostic and medical

centers in Iran University of medical sciences together with a group of 30 normal individuals that had no giardiasis infection as a control. We performed feces examination by direct smear and formol-detergent method and measured GPT γGT and total ALP for liver function. Then we isolated ALP bands by an electrophoretic method. We found that in patients with severe giardiasis infection, there was a rise in the intestinal band of ALP. Many of the samples (77.61%) also demonstrated a rise in bone isozyme as well. Pearson correlation index between giardiasis and intestinal ALP was significant at P= 0.01. The results revealed a clear relationship between giardiasis and the intestinal isozyme of alkaline phosphatase.

p-151
EXTRACTION AND SEPARATION OF LPS FROM THE OUTER MEMBRANES OF HELICOBACTER PYLORI BY SDS-PAGE AND SILVER NITRATE STAINING

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Background: Helicobacter pylori (H. pylori) is one of the major factors of peptic ulcer, gastritis and gastric cancer .One of the properties of this bacterium is its special lipopolysaccharides (LPS) which is responsible for its high resistance against gastric acid and escape from human immune system. This makes it a target for further research or diagnostic goals. For research purposes, extraction and separation of the LPS from the outer membrane of this bacterium is necessary. Methods: LPS were extracted from the outer membrane of H.pylori obtained from patients suffering from gastritis, gastric ulcer and gastric cancer. LPS extraction was done by proteinase K method. SDS-PAGE and silver staining were applied to investigate the electrophoretic pattern of LPS. This pattern was compared with that of E. coli serotype O111: B4 and Salmonella serotype ATCT 14028. Results: The electrophoretic pattern of extracted LPS will be presented to demonstrate the bands in three groups of high, moderate and low molecular weight LPS. Conclusion: One of the major factors in pathogenesis of this bacteria is its LPS. The obtained electrophoretic pattern is ladder shaped, for the different number of oligosaccharide chains. The high molecular weight bands represent S-LPS and the low molecular weight bands represent R-LPS (lack of O-chain).

p-152
STUDIES OF SOME BIOCHEMICALS AND SEROLOGICAL INDICES IN THE SERUM OF BLASTOCYSTIS HOMINIS INFECTED INDIVIDUAL'S

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Background: Blastocystis hominis is an intestinal parasite which was first clearly defined as an organism in 1911 by Allexeieff who proposed the generic name of B. entercola. Although the epidemiologic and biological study of the parasite remains controversial and vague, the prevalence rates in tropical and subtropical and developing and poor countries are increasing. Objective For the first time this study was undertaken to investigate the serum urea, triglycerides, total protein, albumin, alpha1– antitrypsin, and C-reactive protein (CRP) in Iranian subjects infected with B.hominis. Methods: These cross-sectional and case-control studies were performed in 100 adult individuals with B. hominis and 100 healthy individuals without any history of infection referred to health services of Iran University Of Medical Sciences (IUMS) and Milad hospital in Tehran. The biochemical analyses were performed by Cobas Mira auto-analyzer and the Pars Azmoon kits and immune-serologic kits (Binding Site Ltd, Birmingham, UK). Statistical interpretation of data was performed by SPSS version 12 and independent sample t- test. Results: The results of these studies indicated that the mean value of urea, triglycerides and total protein were significantly higher in the B.hominis positive cases than the control grouped. The albumin and CRP values of both groups were not statistically significant but the results of alpha1– antitrypsin were significantly different. Conclusion: It is probable that the protease enzyme secreted by the parasite eliminated the IgA of the protective host and facilitated for the mutualization and localization of B.hominis in the intestinal mucosa. As a result of this phenomenon,, it could cause intestinal inflammation, mal-absorption, and immune defects resulting in an increased value of acute phase proteins (CRP) and alpha1– antitrypsin.

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DETECTION OF FIMBERIA PAP OPERON GENE IN UROPATHOGENIC AND ENTROPATHOGENIC E.COLI (UPEC AND EPEC E.COLI) BY PCR AND COMPARISON WITH PROTEIN PATTERNS BY SDS-PAGE METHOD

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Background and aims: On the basis of many reports , E.coli strains are causative agents for more than 70% of urinary tract infections (UTI). Uropathogenic E.coli is usually isolated from urine whereas entropathogenic E.coli isolated from fecal sample. The aim if present study was detection of Fimberia PAP operon gene in uropathogenic and entropathogenic E.coli isolated from patients with UTI by PCR and comparing it with protein patterns by SDS-PAGE method. Materials and Methods : In this study, 130 E. coli strains were isolated from

130 patients with UTI,. All of the isolated E. coli were serotyped using 13 different O- antisera from UPEC and EPEC strains. Extraction of proteins from isolated E. coli wasperformed by Taylor et al method. A 5-130 Ka molecular weight protein and 2 standard E. coli were used as positive control. The DNA was released from the whole organisms by boiling. Pap1 & pap2 primers were used for PCR, & DNA target segment was 328 bp. Results: Serogroups O6 (23.84%), O18 (12.30%) & O15 (6.93%), were the predominant serogroups of uropathogenic E. coli, respectively. The 44 strains (33.86%) were also entropathogenic E.coli. In this study, 61% of uropathogenic E. coli were positive for the pap operon. Different protein patterns were obtained for uropathogenic and entropathogenic E.coli isolates. Most differences were in 29-45 Kda regions. Conclusion: Our results showed that detection of Fimberia PAP operon gene in uropathogenic and entropathogenic E.coli by PCR was more sensitive than protein extractions (protein patterns) of strains by SDS-PAGE method. We also indicated that predominant serogroups of uropathogenic E. coli were O6 & O18 serogroups in this region.

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BIOCHEMICAL EVALUATION OF TRACE ELEMENTS AND LIVER ENZYMES DURING LEISHMANIA MAJOR INFECTION IN SUSCEPTIBLE BALB/C AND RESISTANT C57BL/6 MICE

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Cutaneous leishmaniasis, caused by Leishmania major is characterized by a skin ulcer. It is widespread through some of urban and rural regions in Iran. Several enzymes which contribute to immunity require Zn and Cu as trace metals for their activity. Their changes are part of organism defense strategies and are induced by the cytokines. Alkaline Phosphatase (ALP), Serum Glutamic Oxaloacetic Transaminase (SGOT) and Pyruvic Transaminase (SGPT) are liver enzymes that are useful for diagnosis of its function and integrity. The purpose of the present study was to compare the Zn and Cu and also the serum concentration of liver enzymes including SGOT, SGPT and ALP in two different susceptible BALB/c and resistant C57BL/6 mice infected with Iranian strain of L. major. In order to carry out this investigation, mice were assigned into 4 groups as control and infected BALB/c and C57BL/6 mice. Experimental leishmaniasis was initiated by subcutaneous injection of the 2×10^6 promastigotes of L. major into the basal tail of infected groups. Serum Zn and Cu were determined by atomic absorption spectrophotometry and serum SGOT, SGPT and ALP were determined by Auto Analyzer RA1000. It is concluded that serum Zn and Cu as essential trace elements were altered during murine CL infection with a different pattern of induction between these two hosts. Alteration of microelements and liver enzymes as a consequence of leishmaniasis may involve in susceptibility and resistance of BALB/c and C57BL/6 hosts against L. major infection.

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IN VITRO LEISHMANICIDAL ACTIVITY OF ELEVEN ARTEMISIA SPECIES OF IRAN

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The genus Artemisia L. is one of the largest and most widely distributed of the Astraceae (Compositae) family, numbering over 400 species in the world. Different species of Artemisia have a vast range of biological effects including antimalarial, cytotoxic, antibacterial, antifungal and antioxidant activity. Leishmania species such as Leishmania major are the protozoan parasites responsible for leishmaniasis. According to the World Health Organization (WHO) while 12 million people are infected by the parasites, 350 million people are living at risk of infection with Leishmania parasites. The annual incidence of new cases is about 2 million from which 1.5 million have cutaneous leishmaniasis. The numbers of cutaneous leishmaniasis is growing in Mashhad, Iran. Thus, the development of a new antileishmanial agent is urgently needed. Previously, leishmanicidal activity of the extract of Artemisia herba alba and ethanol extract of leaves of Artemisia indica was reported but that of other species of Artemisia were not determined. During the search for the candidate anti-parasitic agents from natural resources, we have found that Artemisia species collected from Khorasan province have leishmanicidal activity in vitro. To provide a scientific reason for use of Artemisia species and to obtain 50% inhibitory concentration (IC50) of these extracts, ethanol extracts of eleven species of Artemisia (Astraceae) were examined for their leishmanicidal activity. Some Artemisia species (A. ciniformis, A. santolina and A. kulbadica) showed potent leishmanicidal activity (IC50 25 µg/ml, 80 µg/ml, 25 µg/ml respectively).

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A CLONED ANTIGEN (RECOMBINANT K39SUB) OF LEISHMANIA INFANTUM PRODUCED FOR THE DIAGNOSIS OF VISCERAL LEISHMANIASIS IN DOG

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Surveillance of the canine reservoir is highly important to help control visceral leishmaniasis in humans. It is therefore imperative to improve and develop new reliable, easy to use, and cheap tools for the diagnosis of canine leishmaniasis. K39sub recombinant antigen of Leishmania infantum was expressed in prokaryotic system and evaluated for sero-diagnosis of canine visceral leishmaniasis (CVL). The gene fragment encoding a single 39-amino acid subunit of the kinesin-related protein k39 (k39sub) was amplified from DNA of Iranian strain of L. infantum (MCAN/IR/96/LON49) and

cloned into a pMAL-p2 expression vector in frame with maltose-binding protein (MBP) fusion. The antigenic properties of L. infantum recombinant K39 subunit (39 amino acids) have been tested for the serological diagnosis of CVL by ELISA. K39sub ELISA for CVL was compared with a standard direct agglutination test (DAT) on 55 clinically infected dogs and 71 healthy controls from endemic areas of Ardabil and East Azerbaijan provinces, north-west of Iran. A sensitivity of 72.7% and specificity of 87.3% were found at a 1:320 cut off titer when DAT confirmed cases were compared with healthy control. A good concordance was found between k39sub ELISA and DAT (k= 81.0). Given the antigenic properties shown by the k39sub, we think this protein carries immunodominant epitopes and are valuable for the sero-diagnosis of L. infantum infection in dogs.

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EVALUATION OF SERUM PROTEINS IN GOATS INFECTED WITH THEILERIOSIS USING ELECTROPHORESIS

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Tick- born protozoan parasites of the genus Theileria infect wild and domestic ruminants. Theileria lestoquardi (Theileria hirci) transmitted by Hyalomma anatolicum anatolicum is the causative agent of malignant theileriosis of sheep and goats with a high morbidity and mortality. Blood samples were collected from jugular vein of 8 clinically healthy goats and 18 naturally infected with theileriosis. The serum was separated after centrifugation for 15 min at 750g. Besides clinical signs including pyrexia, lymphadenopathy, ocular and nasal discharge, dyspnea, listlessness, loss of appetite and weight, decline in milk production and finally hypothermia and recumbency with rapid breathing and pulse rate, Theileria lestoquardi was observed in blood smears from infected goats for determination of the disease. A marked anemia was noticed in hematological evaluation of infected goats. Five serum proteins including albumin, α- globulin, beta 1- globulin, beta 2- globulin and gamma - globulin were revealed in cellulose acetate electrophoresis (time: 15 min ; voltage: 180v). There was a significant increase in serum gamma - globulin concentration of infected goats in comparison with clinically healthy ones ($p<0.05$). Gamma - globulin is considered a chronic phase protein and its elevation is due to theileriosis which is a chronic inflammatory disease. A significant decrease in serum beta 1- globulin concentration of infected goats was observed compared to healthy controls ($p<0.05$). The major protein of serum beta 1- globulin is transferrin which is a negative acute phase protein that decreases in inflammations.

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ROLE OF POLYSACCHARIDE ACTIVATORS IN DESIGNING GLYCOCONJUGATE VACCINES

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One of the important strategies in glycoconjugate vaccine preparation is the manipulation of polysaccharide components in order to increase the yield of conjugation. In this study, outer membrane vesicles (OMVs) of Neisseria meningitidis serogroup B and group A meningococcal capsular polysaccharide (GAMP) were obtained by a previously published procedure. The derivatized polysaccharides were activated either with cyanogen bromide (CNBr) or 1-cyano-4-dimetylaminopyridinium tetrafluroborate (CDAP). Then, these activated polysaccharides were conjugated with OMVs by adipic dihydrazide (ADH) and 1-ethyl-3- (3 dimethylamoniumpropyl) carboiimide (EDAC) in order to develop a bivalent glycoconjugate vaccine. The derivatives of activated GAMP-CDAP showed higher yield (45-48%) than GAMP-CNBr activated (15-17%), and both increased the yield of conjugation in comparison with the non activated GAMP (10-12%). . In conclusion, the average yield of conjugation could be improved through a useful activating reagent. CDAP seemed to Bea more useful activating reagent, i.e the treated GAMP had a higher molecular weight and content of O-acetyl than CNBr.

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PREVENTION OF THERMAL AGGREGATION OF α-CHYMOTRYPSIN BY ACETYLATION OF ITS LYSINE RESIDUES

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In the last decade, protein aggregation has moved from a mostly ignored area of protein chemistry, to become a key topic in medical sciences. Accordingly, prevention of aggregation has gained much attention. Chemical modification of proteins has been widely used to enhance protein stability, and has been proven effective against their thermal denaturation. In this investigation, an attempt was made to modify α-chymotrypsin with different degrees of acetylation using acetic anhydride. Therefore with the presence of 14 lysine residues in this enzyme, we expected to observe extensive changes. By increasing the amount of the modifier, we obtained an enzyme with 7 and 8 modified lysine residues and a gradual change of enzymatic activity was obtained as a result. The number of modified lysine residues was determined by the use of fluorescamine. Upon use of increasing concentrations of the modifier, differences in structural properties were observed in the modified forms, compared to the native structure, as suggested by intrinsic fluorescence studies. After heat treatment at 65°C for 30 minutes (pH=8), no aggregates were observed in any of the

modified forms. It is clear that acetylation of lysine residues can prevent thermal aggregation of α-chymotrypsin.

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EXPRESSION OF HUMAN PARECHOVIRUS 2A GENE IN PROKARYOTIC AND IN VITRO SYSTEMS

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Parechoviruses form one of the genera in the family Picornaviridae, and include two human pathogens: Human parechovirus type1 and 2 (Hpev1 and Hpev2).The genome of Picornaviruses encodes a single polyprotein, which undergoes a cleavage cascade performed by virus encoded proteases to give the final virus proteins. The primary cleavage occurs by 2A protein and this step is critical for viral life cycle. Recent sequence analysis suggests that Hpev1 is distinct from other Picornaviruses and lacks the motifs believed to be involved in the protease function of 2A. The aim of this study was to analyse proteolytic activity of 2A protein in Hpev1. For this purpose we made several recombinant plasmids containing 2A region of parechovirus type1 genome and expressed these recombinant plasmids in prokaryotic and in vitro systems under T7 promoter. The products were analyzed by SDS-PAGE, and only one heavy band was detected. Whereas with plasmids including 3C gene, several small bands were observed. In conclusion: the results of this work indicate that Hpev1 has a processing strategy different from other members of Picornaviruses and in this virus, as in hepatovirus, 2A protein does not have a protease function.

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INFLUENCE OF X-IRRADIATION ON DNA ELECTROPHORETIC PARAMETERS OF CANDIDA GUILLIERMONDII YEAST

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The comparative investigation of yeast DNA electrophoretic parameters was realized after X-irradiation at O°C, and after post irradiation repair process in growth medium. The data were compared with those of yeast cells irradiated at room temperature. It was shown that in cases of radiation at low temperature and repaired DNA, in comparison with non-radiated DNA, , there were additional fractions with higher mobility, and a large amounts of not precisely divided fragments with various mobility except for the initial high molecular weight DNA. In case of irradiation of yeast cells at room temperature, irradiated DNA was found only in one fraction, which corresponds to a high-molecular weight DNA and only slightly differed from those for non-radiated DNA. It is concluded that X-irradiation induces a set of structural damages, which are not repaired during the cell irradiation at low temperature, compared the results obtained by X-irradiation of yeast cells at room temperature. The DNA damage induced after X-irradiation of yeast cells are augmented after post-irradiation repair period, perhaps as a result of damaging cell repair system.

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PRACTICAL APPROACH TO TYPING STRAINS OF LEISHMANIA INFANTUM BY ENZYME POLYMORPHISM. A CROSS SECTIONAL STUDY IN NORTHWEST OF IRAN

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In the Mediterranean area, despite their very wide geographical distribution, most Leishmania infantum strains belong to zymodeme MON-1. As different Leishmania species are known to cause different clinical symptoms and may require different treatment protocols, therefore identification of different species of Leishmania is necessary in each endemic site. In order to increase our understanding of enzyme polymorphism of the Leishmania parasites in northwest of Iran, we carried out the isoenzyme analysis of the leishmania parasites isolated from endemic foci of Ahar and Kaleibar districts. In this study, samples isolated from 12 patients (bone marrow aspirates), 26 dogs (splenic and hepatic aspirates) and more than 200 sand flies from Kalybar and Ahar districts between 2004 and 2006. All patients were clinically diagnosed to have visceral Leishmaniasis. Serological profiles of all sera samples from both human and dogs were in accordance with Leishmaniasis (DAT). Isoenzyme profiles of these isolates were compared with those of reference using 12 enzyme systems. L.infuntum. MON-1 is the only zymodeme present in all samples of dogs, sand flies and humans. The enzymatic polymorphism is compared to that of neighboring countries (Azarbaijan, Iraq and Turkey, etc.) and we concluded that the visceral Leishmania (VL) focus in northwest of Iran is evidently of Mediterranean focus of zoonotic VL, which extends from Portugal and Morocco to Pakistan and the central Asian republics. Domestic doges act as the reservoir host, where Phlebotomus kandellakii and perfiliewi ariasi are vectors.

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DEHYDROGENASES ENZYMES AS MARKER OF THE GENETIC STRUCTURE OF KLEBSIELLA PNEUMONIAE

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Electrophoretic variations of dehydrogenases with multilocus enzyme electrophoresis (MEE) have been demonstrated in human beings. An analysis of similar variants in bacteria might provide information for evolutionary and taxonomic

purposes. Dehydrogenase variants might also prove to be useful as genetic markers. The electrophoretic types (ET) characterization of a given enzyme reflects allelic variations at the corresponding structural gene locus, and the combined analysis of metabolic enzymes polymorphism shows genotypes. The aim of this investigation was to analyse the genetic structure of Klebsiella pneumoniae and to consider the relationship between them by dehydrogenase enzymes. 70 specimens were collected from clinical sources of two hospitals in Tehran. Enzymes (proteins extracts) were prepared from bacterial cells by sonication. Electrophoretic mobilities of dehydrogenases including phosphogluconate (PGD), glutamate NAD (GDH), glutamate NADP (GLD), lysine (LyD), isocitrate (ISD) malate (MDH), xanthine (XDH) and glucose 6 phosphate dehydrogenases (G6PD) were detected in starch gel electrophoresis using histochemical staining with their specific substrates, coenzymes and buffer systems by MEE method. Some enzyme loci, such as PGD, GDH (NAD), and G6PD were found to be polymorphic and thus especially informative in epidemiologic studies. Genetic diversity among strains varied from PGD (0.614) to Glucose 6-Phosphate Dehydrogenase (0.387). Mean genetic diversity among these strains was determined as 0.515. The results showed close relationship among strains. Our work revealed that differences in electrophoretic mobilities of some enzymes may reflect the occurrence of point mutation, intergenetic recombination or post translation of proteins. These factors would influence the degree of intra-species heterology.

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DISTRIBUTION OF ALKANE HYDROXYLASE GENES INTO CRUDE-OIL DEGRADING BACTERIAL POPULATION IN PERSIAN GULF

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Crude-oil bio-reclamation is an efficient and economical method for remove of oil pollution in marine environments. Rate of bio-reclamation depends on concentration of crude-oil, length of alkanes and kind of microorganisms. Over than 60 % of crude-oil component are alkanes. Alkanes with medium length (C10-C20) are favorable substrates for degradation. Alkane hydroxylase enzyme is a key enzyme in alkane degradation. This enzyme introduces an oxygen atom into alkane substrate and has an important role in petroleum bioremedy. Alkane hydroxylase gene is divided into two phylogenic groups. Group (II) alkane hydroxylase genes encode enzymes that degrade medium chain alkanes (C6-C12) and Group (III) alkane hydroxylase genes encode enzymes that degrade long chain alkanes (up to C12). Various contaminated sediments in Persian Gulf were collected. Isolation of crude-oil degrading bacterial was done in basal medium supplement with 1 % crude-oil as sole source of carbon and energy. After three subcultures, colonies were purified on oil agar medium. DNA was extracted from strains by miniprep method and PCR performed with primers of Group (II) and Group (III) alkane hydroxylase genes. Fifty crude-oil degrading bacterial strains were isolated. Most strains belonged to Pseudomonas, Acinetobacter and Rhodococcus genus. Thirty strains had a 330bp PCR product by amplification with Group (III) primers and fifteen strains had a 270 bp PCR product by amplification with Group (II) primers. Five strains did not have any PCR product. Group (III) gene had 60 % frequency and Group (II) had 30 % frequency in Persian Gulf ecosystem. Ten percent of frequencies belong to strains that have Group (II) and Group (III) genes. Group (III) alkane hydroxylase gene encodes enzymes with substrate range up to C12 and this is in accord composition of oil pollutants in Persian Gulf. Because of evaporation, alkanes with low chain are degraded and long chain alkanes remain intact. Such conditions induce selective effects on the prevalence of Group (III) alkane hydroxylase gene. But presence of bacteria with two alkane hydroxylase genes is of interest and indicates that bacteria can grow on both medium and long chain alkanes. Presence of bacteria without any alkane hydroxylase indicates that these bacteria use other systems for biodegradation of petroleum hydrocarbons.

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GENETIC VARIATIONS OF PASTEURELLA MULTOCIDA AS DEMONSTRATED BY 16S-23S RRNA GENE SEQUENCE COMPARISON

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Pasteurella multocida is known as an important heterogenic bacterial agent causing some severe diseases such as fowl cholera in poultry and haemorrhagic septic anemia in cattle and buffalo. A polymerase chain reaction (PCR) assay using primers derived from conserved part of 16S-23S rRNA gene was developed. The PCR amplified a fragment of 0.7 kp size using DNA from eight avian P. multocida isolates .Sequence alignment of the 16S-23S rRNA genes revealed a considerable heterogenicity among the isolates. The percent of similarity varied from 84 to 99.1 among the isolates .An interesting finding from this study was the presence of a repeat sequence (seven mer) in the 16S-23S rRNA region in 50% of the isolates. According to presence or absence of this repeat sequence, the P .multocida isolates are classified into 2 distinct clusters. The virulence of cluster I isolates was higher than cluster II isolates. Ribotyping of P. multocida using 16S-23S rRNA gene PCR sequencing could be used for the construction of an epidemiological marker

Biophysical-Chemistry

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NOVEL TWO-STAGE HYBRID NEURAL DISCRIMINANT MODEL FOR PREDICTING STRUCTURAL CLASSES OF PROTEINS

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In order to establish novel hybrid neural discriminant model, linear discriminant analysis (LDA) was used to evaluate the contribution of sequence parameters in determining the structural class of protein. An in-house program generated

parameters including single amino acid and all dipeptide composition frequencies for 498 proteins came from G. P. Zhou [An intriguing controversy over protein structural class prediction, J. Protein Chem. 17(8) (1998) 729–738]. Then, 127 statistically effective parameters were selected by stepwise LDA and were used as inputs of the artificial neural networks (ANNs) to build a two-stage hybrid predictor. In this study, self-consistency and jackknife tests were used to verify the performance of this hybrid model, and were compared with some of prior works. The results showed that our two-stage hybrid neural discriminant model approach is very promising and may play a complementary role to the existing powerful approaches.

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ELECTROCHEMICAL BEHAVIOR OF REDOX PROTEINS IMMOBILIZED ON RIBOFLAVIN-NAFION FILM MODIFIED GOLD ELECTRODE

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Electron transfer of a redox protein at a bare gold electrode is too slow to observe the redox peak. A novel Nafion-Riboflavin functional membrane was constructed. Electron transfer of cytochrome c, superoxide dismutase, and hemoglobin was carried out on the functional membrane modified gold electrode with good stability and reproducibility. The immobilized protein modified electrodes showed quasi-reversible electrochemical redox behaviors with formal potentials of 0.150, 0.175, and 0.202 V (versus Ag/AgCl) in 50 mM MOPS buffer solution, pH 6.0 at 25^{o} C. The cathodic transfer coefficients were 0.67, 0.68, 0.67 and electron transfer rate constants were evaluated to be 2.25, 2.23 and 2.5 $s^{-1.}$

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IN VITRO EVALUATION OF ANTIOXIDANT AND FREE RADICAL SCAVENGING ACTIVITIES OF DIFFERENT FRACTIONS OF ANETHUM GRAVEOLENS

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Anethum graveolens L. (dill) [umbelliferae] is used in Iranian folk medicine as an anti-inflammatory drug. since this activity may be correlated with the presence of antioxidants, the antioxidant potency of leaf crude extract and different fractions (diethyl ether, ethyl acetate and water) were investigated, employing various established in vitro systems, such as the ferric reducing antioxidant power (FRAP) and 2, 2'azinobis 3-ethylbenzothiazoline-6-sulfonate (ABTS+) assays, 1,1-diphenyl-2-picrylhydrazyl (DPPH) /nitric oxide radical scavenging, and iron ion chelating activity. Total phenolic and flavonoid contents of different fractions of A. graveolens were also determined by a colorimetric method. In the above assays, crude extract and all the fractions showed antioxidant potential to varying degrees. Among these fractions, ethyl acetate fraction exhibited higher antioxidant potency compared to the other fractions, which was correlated with its total phenol and flavonoid content. The data obtained from the in vitro models clearly established that A. graveolens especially ethyl acetate fraction has antioxidant and free radical scavenging activities.

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A THERMODYNAMIC STUDY OF CALCIUM, MAGNESIUM AND COBALT IONS BINDING TO MYELIN BASIC PROTEIN

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The interaction of myelin basic protein (MBP) from bovine central nervous system with three divalent metal ions (Mg^{+2}, Ca^{+2} and Co^{+2}) was studied by isothermal titration calorimetry, ITC, at 300 K in aqueous solution. The extended solvation model was used to reproduce the enthalpies of metal ion-MBP interaction. The solvation parameters recovered from the solvation model were attributed to the structural change of MBP due to the metal ion interaction. It was found that there is a set of two, and three identical and noninteracting binding sites for Mg^{+2}, Ca^{+2} and Co^{+2} ions, respectively. The molar enthalpy of binding for Mg^{+2}, Ca^{+2} and Co^{+2} was -15.1, -15.4 and -14.5 kJ mol-1, respectively. The association equilibrium constant for Mg^{+2}, Ca^{+2} and Co^{+2} is 48, 42 and 63 μM-1, respectively. We have previously developed a theory to account for the solvation of solutes in mixed solvent systems. Studies within our group are aimed at developing an understanding of how the metal ions and other ligands binding to proteins affect on the stability of the biomolecules. One of the unique aspects of our approach is studying the stability of proteins by using the extended solvation model. As a clear understanding of operational stability constitutes, an important goal in protein technology, our efforts aimed at elucidation of the structure-stability using the extended solvation model. This model is able to correlate the solvation parameters to the effect of metals on the stability of a protein in a very simple way. One important posttranslational of MBP that correlates with the severity of autoimmune disease multiple sclerosis (MS) is deimination, the enzymatic conversion of arginine to citrulline by peptidylarginine deiminase. Deimination limits MBP ability to maintain a compact myelin sheath by disrupting both its tertiary structure and its interaction with lipids. Ca^{+2}-MBP, Mg^{+2}-MBP interactions increase the stability and the biological activity of MBP and Co^{+2}-MBP interactions decrease the stability and the biological activity of MBP.

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AN INHIBITION STUDY ON MUSHROOM TYROSINASE BY ALKYL XANTHATES

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Two groups of branched and nonbranched of seven n-alkyl xanthate compounds, as sodium salts were examined for inhibition of both cresolase and catecholase activities of Mushroom Tyrosinase (MT) from a commercial source of *Agricus bisporus*. The assay was performed in 10 mM phosphate buffer pH 6.8, at 293 K using UV spectrophotemetry. 4-[(4-methylbenzo)azo]-1,2-benzendiol (MeBACat) and 4-[(4-methylphenyl) azo]-phenol (MePAPh) were used as synthetic substrates for the enzyme for catecholase and cresolase reactions, respectively. Lineweaver-Burk plots showed different patterns of mixed, competitive, uncompetitive and noncompetitive inhibition for seven xanthates and also for cresolase and catecholase activities of MT. In nonbranched and branched alkyl xanthates the Ki value increases by increasing the length of the alkyl chain in catechoase activity without any significant difference in Ki values in the cresolase activity for both groups. Nonbranched compounds can inhibit both activities of the MT better than branched alkyl xanthates. Both groups of compounds are activators of cresolase activity of the enzyme at low concentrations. The inhibition of cresolase is related to the chelating of the copper ions at the active site by a negative head group (S̄) of the anion xanthate, which leads to similar values of Ki for xanthates in each group. Different Ki values in catecholase inhibition are related to different interactions of the aliphatic chains of seven xanthates with hydrophobic pockets in the active site of the enzyme.

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STUDY ON THE INTERACTION OF SODIUM DODECYL SULFATE AND DODECYLTRIMETHYLAMMONIUM BROMIDE WITH LYSOZYME BY ISOTHERMAL TITRATION CALORIMETRY

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Thermodynamics of the interaction between Sodium Dodecyl Sulfate (SDS) and Dodecyltrimethylammonium Bromide (DTAB) with lysozyme were investigated at pH = 7.0 and in phosphate buffer by isothermal titration calorimetry. A new method to follow protein denaturation, and the effect of surfactants on the stability of proteins was introduced. The new solvation model was used to reproduce the enthalpies of lysozyme-surfactant interaction over the whole range of SDS and DTAB concentrations. The solvation parameters recovered from the new equation, attributed to the structural change of lysozyme and its biological activity. It is possible to use this equation to reproduce the enthalpies of lysozyme-surfactant interaction as follows: where Q is the heat of lysozyme-surfactant interaction at certain ligand concentrations and Q represents the heat value upon saturation of all lysozyme molecules by surfactant. and are the local mole fractions of the components A and B in the vicinity of the solute or solvation sphere. and are the net effects of the solute on solvent-solvent bonds in water-rich domain and cosolvent-rich region, respectively. and are the relative partial molar enthalpies. and are bulk mole fractions. is the enthalpy of denaturation. is the fraction of denatured lysozyme, which can be expressed as follow: and are heat of lysozyme in the native and denatured states, respectively. At low concentrations of SDS, the binding is mainly electrostatic, with some simultaneous interaction of the hydrophobic tail with nearby hydrophobic patches on the lysozyme. These initial interactions presumably cause some protein unfolding and expose additional hydrophobic sites. More SDS molecules then bind to the expose hydrophobic sites in the lysozyme-SDS aggregate. The enthalpies of denaturation of lysozyme are 159.62 and 87.087 kJ mol for SDS for DTAB, respectively.

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CHANGE IN REORGANIZATION ENERGY DURING CYT C UNFOLDING

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So far, the protein unfolding/refolding has been mainly studied by spectroscopic methods. In this report we have tried to describe this process in metalloproteins by an electrochemical variable so called "reorganization energy" (λ). Reorganization energy is one of the most fundamental parameters in electron transferring process. It is the energy that is required for configurational change of a redox species so that the electron transfer could takes place. By exposing the redox centre to a dynamic environment, λ increases and electron transfer rate constant decreases. So, in metalloproteins, the exposure of redox centre to the solvent causes an increasing in λ. Since, heame egression/ reentrance in protein unfolding/refolding process have already been reported in the literature; λ could be an independent and sufficient criterion for monitoring the heamoproteins folding/refolding. In the present work, the unfolding of cyt c induced by urea was studied by cyclic voltammetry. In each concentration of urea, λ of intermediate state of cyt c was measured and at last reorganization energy was plotted versus urea concentration. The stability of protein which extracted from this curve was 8.5 kcal/mol, which is comparable with those reported in literature.

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ELECTROCHEMICAL STUDIES OF THE PEROXIDASE MODIFIED BY ANTHRAQUINONE

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Measurement of H2O2 has attracted considerable interest because of its very crucial role in biomedical and environmental studies as well as industrial process. Hydrogen peroxide also is involved in many biological reaction as a by product of several oxidases, and its measurement account for substrate level in enzyme assay. In the present report we used

an enzyme modified electrode to determine the concentration of H2O2 in solution. We used anthraquinone 2 carboxylic acid, as an electron relay for horseradish peroxidase (HRP). Covalent attachment of this relay to the enzyme structure also promoted physical adsorption of HRP on the glassy carbon electrode. Immobilization was stable and functional for 20 days. Cyclic voltammetric and chronoamperometric results proved this modified electrode as a stable and functional biosensor. Detection limit of this biosensor was measured to be 18 μM and the responses were linear up to 9.0 mM/L. The response time was 5 seconds and biosensor can be used repeatedly in storage at 4^0C for at least 20 days with a little deterioration in response.

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IMMOBILIZATION OF HORSERADISH PEROXIDASE USING TOLUIDINE BLUE-NAFION FUNCTIONAL MEMBRANE

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An immobilization approach based on the electrostatic and entrapment of horseradish peroxidase (HRP) in a functional membrane of toluidine blue-nafion was investigated. The structure of the functional membrane was characterized by UV-Vis and Fourier Transform Infrared techniques. This showed that additions of toluidine blue to nafion promote the formation of its dimmer form and there is bounding interaction between the –SO3- of nafion and the aromatic ring of toluidine blue. The electrochemical measurements were employed to check for any loss of the activity of the enzyme during the immobilization. The results showed that the immobilized enzyme has a good sensitivity to H_2O_2. It can function as an electrochemical sensor for measuring H_2O_2. The linear range of this biosensor was from 1×10^{-5} to 5×10^{-3}M, with detection limit of 5×10^{-6} M.

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EFFECT OF DEFERASIROX ON STRUCTURE AND FUNCTION OF HEMOGLOBINS A AND β-THALASSEMIA

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Transfusional iron overload is a major cause of morbidity and mortality in thalassemia, sickle-cell disease, and other chronic anemias. Iron overload can be effectively managed by adequate chelation therapy as documented by experience with deferoxamine (Desferal®). However, treatment with deferoxamine has shown negative effect on many patients due to the regimen of parenteral infusions which in over an 8–12-hour period, 5–7 times a week. Poor compliance to deferoxamine therapy is even more pronounced among adolescents. Deferasirox (Exjade®, ICL670) is a new class of tridentate iron chelators that functions as an orally active iron chelator. Deferasirox can interact with proteins. One of the proteins which could be targeted for this drug is hemoglobin. Interaction of deferasirox with hemoglobin has not been studied before. In this study, we investigated the interaction of deferasirox with normal (HbA) and major β-thalassemia hemoglobins. The structural change of hemoglobins A and β-Thalassemia upon interaction with deferasirox were studied by circular dichroism (CD) spectropolarimetry and oxygen affinity of aforementioned interaction investigated by sodium dithionite that changes the partial pressure of oxygen in solution. Results shown deferasirox doesn't effect on structure and function of normal and β-thalassemia hemoglobins with concentration equivalent to clinical dose, but alters the oxygen affinity and structural conformation of hemoglobins by overdose concentration.

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AMYLOID FORMATION AND SURFACE ACTIVITY RELATIONSHIP OF GLYCATED HUMAN SERUM ALBUMIN BY DIFFERENT SUGARS

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Glycation is a spontaneous modification of proteins in which reducing sugars bind covalently to the free amino groups. This process finally produces heterogenic products that are collectively called advanced glycation end products (AGEs) and also induce aggregation of proteins that are the cause of several conformational diseases such as Alzheimer and Parkinson. Here we studied the ability of three reducing sugars (glucose, fructose and ribose) in the glycation process on human serum albumin (HSA), generation of amyloid structures, and detergent-like effects of AGEs as well as amyloid formation by changes in surface activity. The fluorescence assessment, surface tension analysis, and electron microscopy were utilized to evaluate the structures of glycated HSA. Incubation of HSA with aforementioned sugars after 20 weeks resulted significant amyloid fibril formation that revealed by transmission electron microscopy (TEM). Glycation of HSA is in the direction of transition from a helical structure to ß-sheet and amyloid formation. In this regard, the physicochemical properties, such as excess free energy, protein adsorption at the air-water interface, critical aggregation concentration (CAC), and surface activity obtained and results indicate hydrophobicity enhancement and partial unfolding. The prolonged glycation of HSA has shown its significant effects on structural and physicochemical properties of protein which is influenced by the type of sugar involved.

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OXIDANT/ANTIOXIDANT INDICES IN OCULAR INJEURIES INDUCED BY SULFUR MUSTARD IN AN ANIMAL MODEL

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Introduction: exposure to sulfur mustard (SM) produces ocular impairment. Despite researches done in this field, the SM mechanism in inducing ocular injures is unclear. Because of the ocular inflammatory effects of SM and the role of oxidative stress in inducing ocular inflammatory disease, we decided to study the aquause H_2O_2 concentration as an oxidant and super oxide dismutase enzyme (SOD) activity as antioxidant indices in animal model of ocular injuries induced by SM. Materials and Methods: Distinct amounts of NM and SM administered in the left eye of 6 six rabbits, respectively. Two hexad groups of rabbits without any exposure with the SM and NM, selected as control groups. One and five day after exposure aquase sampling was done. H_2O_2 concentration and SOD activity measured in specimens. ANOVA statistical test was used for comparison between groups. Results: Aquase H_2O_2 concentration was lower significantly in the specimens of first and 5th day in SM exposure group in comparison with control group. But SOD activity was higher significantly in the specimens of only 5th day specimen. There were no differences between measured indices in NM group in comparison with control group. Discussion: According to the gradual increase in SOD activity and decreased concentration of H_2O_2 in aquase specimens of rabbits exposed to SM, H_2O_2 can be considered as an oxidant reactant in inducing ocular complications of SM in early period after exposure. Regarding the slow process of increasing SOD activity, it can inhibit H_2O_2 effects in early period after exposure.

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STUDY OF PROTEASE-SENSITIVE REGIONS IN LUCIFERASES FROM GLOW-WORM LAMPYRIS TURKESTANICUS AND FIREFLY PHOTINUS PYRALIS

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Luciferase has been used as a useful reporter protein due to its simple detection and its quick response to change in transcription. Firefly luciferase catalyzes the formation of a bound luciferin-AMP, which then reacts with O_2 to generate oxyluciferin-AMP in an excited state. Breakdown of this intermediate to oxyluciferin, CO_2, and AMP is accompanied by emission of a photon. One of its distinctive properties is a pronounced susceptibility to proteolytic in vitro and very short half-life in vivo. To define the structural basis for this behavior, limited proteolysis studies were undertaken using trypsin and chymotrypsin to identify region of the protein which are sensitive to cleavage. Firefly luciferase incubated at different temperatures with trypsin and chymotrypsin for varying lengths of time. Then the reaction mixtures were subjected to SDS-PAGE electrophoresis. Cleavage with chymotrypsin in P. pyralis luciferase and L. turkestanicus luciferase yielded similar product pattern but cleavage with trypsin showed different digestion pattern. Digestion of P. pyralis luciferase yielded an initial product of about 40 KD that was left with time. The results of proteolysis also showed that proteolytic sensitivity in L. turkestanicus luciferase is more than P. pyralis luciferase.

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PROGRESSIVE HEME OXIDATION DURING GLYCATION OF HEMOGLOBIN

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Non-enzymatic glycosylation or glycation is a process in which reducing sugar(s) react spontaneously with amino groups in proteins to produces advanced glycation end products (AGEs). This phenomenon introduces considerable alteration on the structure of proteins including hemoglobin (Hb). The nonenzymatic reaction between moderately reactive reducing sugar; fructose and globin chains from metHb resulted in hemin release and degradation which are documented through photometric and fluorometric spectroscopy. In view of the glycation of metHb by fructose extensive hypochromic effect was resulted along with a small bathochromic effect in Soret region (410 nm). However slight hyperchromic effect was also observed in UV region due to the structural alteration of the apo-portion of the metHb during 14 days of the fructation process. Hemin degradation products during the formation of Hb-AGE were also monitored using fluorescence method, and two fluorescence bands were measured. Fluorescence intensity of these products increased with increasing of incubation time. It is presumed that fructation-induced hemin release make it more accessible for oxididative degradation via a complicated reactive oxygen species involved process.

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PURIFICATION OF CAMEL ALPHA-LACTALBUMIN AND COMPARISON OF ITS THERMAL STABILITY WITH BOVINE ALPHA-LACTALBUMIN

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After defating of camel milk, the casein fraction was precipitated using ammonium sulfate (26.4 %) and the supernatant was applied on an UF membrane 30 kDa. The filtrate collected and was further concentrated on another UF membrane with different cut off (10 kDa). The pure sample of camel α-Lactalbumin (α-la) which retained by 10 kDa UF membrane was colleted and dialyzed against double distilled water and then lyophilized. Differential scanning calorimetry (DSC) was used as a sensitive tool for measuring the protein thermal stability. Thermal denaturation of both camel and bovine α-lac was found to be reversible in 20 mM Tris (pH 7.5). The transition temperature (Tm) of camel and bovine α-lac was found to be 72.1 and 64.4 ºC, respectively. Far-UV circular dichroism (CD) also showed that camel α-lac is more stable against thermal denaturation. However, the camel secondary structure contains more beta structure and less random coil and helix than bovine α-lac.

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COMPARATIVE STUDY OF STRUCTURAL CHANGES OF BETA CASEINS UNDER THERMAL STRESS

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The caseins are the most important and basic milk protein, which play a major function in micelle form to transport the indispensable human nutrient insoluble calcium and phosphate ions. The goal of this work was exploration of the role of conformational changes under thermal changes on the chaperone activity and calcium transfer ability of beta-caseins (bovine, camel and goat) and theirs allergenicity. Based on the controversial assumption that beta-casein has a random coil and flexible structure similar to "rheomorphic" proteins, their crystallographic study is impossible. In this study, surface tensiometry, viscometry, circular dichroism (CD), fluorescence and UV-Vis absorption spectroscopy have been used for the monitoring of the structural changes of beta casein on the thermal stress. The provided results describe the structural and physicochemical similarities and dissimilarities of beta-caseins of different sources. Also the contributions of self-association and conformational changes in the observed experimental results have been studied. Results show that beta-casein may fold considerably prior to self-association, as well as structural changes occurred after maturity of association. The 1-anilino-8-naphthalenesulfonate (ANS) extrinsic fluorescence and CD spectra are an evidence for the creation of a partial unfolded form of beta-casein. This might be the promoter of self-association and formation and stabilization of casein micelles.

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CAMEL αS1-CASEIN: THERMAL BEHAVIOR OF PHOSPHORYLATED AND DEPHOSPHORYLATED STATES

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Alpha S1-Casein (αS1-CN) is the most abundant protein component in both camel and bovine milk. αS1-CN has been reported to be most allergenic protein component of the bovine milk. Dephosphorylation of caseins is highly desirable because the phosphate groups of caseins are known to affect many characteristics such as casein digestion, bioavailability of divalent cations and allergenic reactions. In this study attempt to compare thermal behavior of intact camel αS1-CN and its fully dephosphorylated species. Camel αS1-CN was purified, using diethyl amino ethyl (DEAE) cellulose as the anion exchanger matrix and the purity of the protein sample was analyzed using SDS-PAGE and urea PAGE. Potato acid phosphotase was used to remove the phosphate groups of the casein and degree of dephosphoryalation was assessed by using different techniques, including SDS-PAGE, Urea-PAGE and an electrochemical method. The thermal profile of the protein in phosphorylated and totally dephosphorylated states was monitored by following the change of intrinsic fluorescence intensity under forward- and backward temperatures between 10 to 90 ºC. The changes of maximum fluorescence intensity of the intact protein demonstrated an entirely reversible nature whereas in the dephosphorlated form camel αS1-CN showed an irreversible thermal profile. On the base of this finding it could be suggested that heating-cooling process is unable to remove any possible conformational epitope of the intact protein whereas antigencity/allerginicity of this protein in dephosphorylated state seems to be more removable under heating stress. This proposition is needed to be confirmed through further research, using Enzyme-Linked Immunosorbent Assay (ELISA).

Enzymology

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SEPARATION AND PARTIAL CHARACTERIZATION OF N-ACETYL-β-D GLUCOSAMINIDASE FROM CHICKPEA SEEDS.

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Abstract: A soluble N-acetyl-β-D-Glucosaminidase from Chickpea seeds were purified by conventional methods of protein isolation. Analytical electrophoresis of the purified preparation in 12% SDS–polyacrylamide gels stained with Coomassie blue and silver staining revealed two non-identical subunits of 14.1& 11.9 kDa that was responsible for enzyme activity. The KM and Vmax values for p-nitrophenyl-N-acetyl-β-D-glucosaminide were determined to be 45.5 µM and 741.8 micromol min (-1) mg(-1), respectively. The purified enzyme is a glycoprotein with 3.1% carbohydrate. Optimum pH and temperature of the enzyme activity were 4.8 and 40^{o} C. Molecular weight of pure enzyme by SDS PAGE and gel filtration were 26.0 and 26.3 KDa respectively.

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STUDY OF THE ACTIVITY OF OMP DECARBOXYLASE FROM PLASMODIUM FALCIPARUM: SYNTHESIS OF THE SUBSTRATE AND AN INHIBITOR OF THIS ENZYME

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Despite more than a century of efforts to control malaria, the disease remains a major and growing threat to public health. Malaria kills approximately one million people annually. The exploitation of biochemical differences between the parasite and the host offers a rational approach to the development of new anti-parasitic drugs. The last two steps of de novo UMP

biosynthesis are catalyzed by the enzymes orotate phosphoribosyltransferase (OPRTase) and OMP decarboxylase (ODCase). ODCase is a target for development of anti-malarial drugs that would be active against drug-resistant parasites. A variety of assays are available for ODCase including spectrophotometric and measurement of 14CO2 released from [carboxyl-14C]OA or OMP. [2-14C]OMP and 6-Azauridine 5'-monophosphate is not commercially available, and was synthesized enzymatically. The kinetic analysis of the malarial ODCase shows that the dependence of enzymic activity on [OMP] follows Michaelis-Menten kinetics. Inhibition studies of the pfODcase provide evidence for the mechanisms by which various drugs interfere with the de novo pyrimidine biosynthetic pathway. Kinetics of inhibition, using a nucleoside 5'-monophosphate inhibitor was examined. To define the Ki value of 6-azaUMP for pfODCase, a range of 6-azaUMP concentrations was examined at a fixed concentration of [2-14C] OMP. The data show that inhibition by 6-azaUMP is linear competitive with respect to OMP. The parameters were determined from the initial velocities obtained at appropriate OMP and 6-azaUMP concentrations. The inhibitory effect of 6-azaUMP shows that it binds to pfODCase 10 times more tightly than to human erythrocyte ODCase.

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BROILER LIVER AS A GOOD MODEL FOR DETOXIFICATION MECHANISMS: HISTOPATHOLOGICAL AND HISTOCHEMICAL STUDY OF THE EFFECT OF CARBON TETRACHLORIDE ON BROILER LIVER

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Introduction: There are a few indications that the avian liver shows more resistance than the mammalian liver against common hepatotoxins such as CCl_4. Certain factors such as high growth rate and increased metabolism make broilers good candidates for toxicological research. Methods: Male broilers (Arian strain) at ages 40 and 25 days were divided into 7 groups as follows: oral treatment with 0.5, 1, and 2.5 cc/Kg B.W. of CCl_4 (age 40 days); oral treatment with 2.5, and 4 cc/Kg B.W.CCl_4 and IP injections of 2.5 cc/Kg B.W. CCl_4 (age 25 days), and the control group. During the experimental period, daily weight gain and the behavior of broilers in each group were recorded. At the end of the experiment we stained the liver samples by H.E and PAS for pathological and histochemical studies. Results: Our findings show that oral treatment with CCL4 (0.5, 1, and 2.5 cc/Kg B.W.) in 40- day old broilers; and oral administration of CCl4 (2.5, and 4 cc/Kg B.W.) and IP administration of 2.5cc/Kg B.W. in 25- day old broilers could not induce any clear hepatotoxicity such as fatty degenerative changes or necrosis in the liver. Also, histochemical studies did not show any significant difference between glycogen storage in experimental and control groups. Conclusion: It is suggested that broilers show more resistance against hepatotoxins than rats or other mammals. Treatment with toxic doses of CCl4 results in centrilobular necrosis in the rat , while in broilers, treatment with higher doses of this hepatotoxin reduces the daily weight gain without any significant hepatotoxicity. It seems that broilers have a very strong detoxification system, even at young age, which can be a result of high auto protection mechanisms such as reduced gap junctions, increased tissue repair systems and different glutathione cycle in their hepatocytes .Based on this research we suggest that the liver of broilers could be used as a good model in in vivo and in vitro hepatotoxicity investigations.

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ADSORPTIVE IMMOBILIZATION OF LACTOPEROXIDASE ON CAJANUSE CAJAN LECTIN VIA CARBOHYDRATE MOIETIES

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Lactoperoxidase (LPO) belongs to the innate defense factors in human milk, saliva and tears. LPO has been used as a preservative in food and pharmaceuticals as well as in oral health care products to restore antibacterial capacity of saliva in patients with dry mouth. The immobilization of enzyme is an important goal in chemical, pharmaceutical and food industries as well as in clinical and chemical analysis, because it offers several advantages including enzyme reuse, ease in product separation, improvement in enzyme stability and continuous operation in reactors. Thus extensive studies have been made in the last three decades to obtain an immobilized enzyme. Cajanuse cajan lectin (CCL) was coupled to the cyanogen bromide activated Seralose. To 1ml of activated matrix was added 1ml of 4mg/ml lectin in the coupling buffer (0.1M sodium bicarbonate buffer, pH 8.5). LPO (26ug/ml) was incubated for 12 h at 40 C and with 1ml CCL_Seralose 4B in a total volume of 50 mM phosphate buffer pH 7 containing 0.15M NaCl, 1 mM CaCl2, and 1 mM MnCl2. The unbound enzyme was removed by centrifugation and repeated washing with the same buffer. The optimum temperature and pH of the enzyme did not change upon immobilization .The immobilized and free enzymes were kept in different pH buffer solutions for 60 min. The immobilized enzyme was stable from pH 4 to 8, while free enzyme was stable from pH 6 to 7. The immobilized and free enzymes were kept at different temperature from 10 C to 80 C, for 60 min. The immobilized enzyme was more stable than free enzyme .The long-term stability of immobilized and free enzyme was investigated. At 40 C the immobilized enzyme retained almost 80% of its activity after 10 days and almost no activity was observed on day 60, and the free enzyme was inactivated on day 9. At room temperature the immobilized enzyme activity was stable up to 7 days and retained almost 10 % of its activity after 30 days, but free enzyme was inactivated on day 5. The Km of the enzyme increased from 0.065 to 0.071 mM upon immobilization. This agrees well with the results from immobilization of invertase on CCL-Seralose 4B. Thus immobilized enzymes may have an industrial potential for development of a biosensor for glucose determination in blood and other sources.

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EFFECT OF CATIONS ON THE AFFINITY OF LDH-C4 FOR PYRUVATE

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Introduction: LDH –C4 is a specific isoenzyme district from other LDH isoenzymes with regard to its locality and kinetic properties. About 80% of of LDH in spermatozoa contains LDH-C4, therefore it must be strongly related to very specific metabolic process connected with sperm. Aim: In the present research the kinetic properties of LDH-C4 extracted from mouse testis was studied. Methods: In order to investigate the kinetic properties of LDH-C4 isoenzyme, testis were removed from epididim of 40 adult rat and homogenized in a potter Elvejhem homogenizer with one part of distilled water and centrifuged at 20000x g for 20 min in a refrigerated centrifuge at 4O C. By hemogenation, ammonium sulfate precipitation and DEAE Sephadex ion exchange chromatography enzyme was extracted. LDH-C4 activity was determined by recording the absorbance change at 340 nm produced by the oxidation of NADH. Protein was measured according to Lowry procedure. The homogeneity of enzyme was proved by polyacrilamide gel electrophoresis. Effect of pH, temperature, pyruvate concentration on the activity of LDH was studied. Results. After electrophoresis and staining for lactate DH, a single band of activity was revealed. Optimum pH was 7.4. Enzyme had 80% of activity at 60 OC for 15 min. When pyruvate used as substrate , optical density increased up to 0.2 uM and then decreased and substrate had inhibitory effect. Lineweaver & Burk plot showed Km of 0.031 mM for pyruvate. Specific activity of eluates was 42.6 U/mg and purity attained by this method is 71 fold. Conclusion: In this work we could purify LDH C4 from rat testis.This enzyme is related to metabolic process that provide energy for mobility and survival of spermatozoa, therefore it can be use for antifertility studies in our future reasearch. Key Words: LDH-C4, Kinetic, extraction.

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CHARACTERIZATION OF ALCOHOL DEHYDROGENASE PURIFIED FROM GLUCONOBACTER SUBOXYDANS

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Alcohol Dehydrogenase (ADH) of acetic acid bacteria, catalyzes the first step of acetic acid production that is the oxidation of ethanol to acetaldehyde. These bacteria, Acetobacter and Gluconobacter, are able to oxidize ethanol to acetic acid by two sequential catalytic reactions of ADH and aldehyde dehydrogenase. ADH is a membrane bound enzyme that was purified in this study from Gluconobacter suboxydans. The enzyme has been solublized by Triton X-100 and fractionated on DEAE-Sephadex A-50 and hydroxyapatite. The purified enzyme was characterized and enzyme properties were discussed. The optimum pH of the enzyme was 5.5-6.5 , and the optimum stability pH was at 6-9. Substrate specificity of this enzyme, as a typical primary alcohol dehydrogenase, for ethanol is higher than other substrates. An apparent Michaelis constant for ethanol was examined to be 1.7×10^{-3} M.

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IDENTIFICATION OF HISTIDINE RESIDUES AT THE ACTIVE SITE OF RAT LIVER PLASMA MEMBRANE PHOSPHATIDATE PHOSPHOHYDROLASE

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Phosphatidate phosphohydrolase (PAP) catalyzes the dephosphorylation of phosphatidic acid to yield Pi and 1,2 diacylglycerol .The diacylglycerol serves as an immediate precursor for the synthesis of major glycerolipids in animal cells. This reaction is a regulatory step in the synthesis of triacylglycerol and phospholipids. Two different forms of PAP in rat hepatocyte have been reported. PAP1 is located in cytosolic and microsomal fractions and participates in the synthesis of phospholipids and triacylglycerols, whereas the other form of phosphatidate phosphohydrolase (PAP2) is primarily involved in lipid signaling pathway by modulating the second messenger diacylglycerol and phosphatidic acid. In addition, PAP2 has two ioforms,PAP2a and PAP2b. PAP2b was purified from rat liver plasma membrane by n-octyl glucoside , Triton X-100 and several chromatography steps. The enzyme was inactivated by diethylpyrocarbonate (DEPC) and the number of mol of histidine residues modified per mol enzyme was determined . In order to distinguish substrate protection against the inhibitory effect of DEPC, the enzyme was incubated with substrate and DEPC. The specific activity of purified enzyme was 3750mU/mg protein and it showed only a major single band on SDS-PAGE with a MW of about 33.8 kDa. Gel filtration experiment showed a MW of 182 kDa for native purified PAP2b. The preincubation of PAP2b with DEPC inhibited the enzyme activity by 90 % due to covalent modification. Six histidyl residues were modified per mole of the enzyme when about 90% of the enzyme activity was lost.The treatment of DEPC-inactivated PAP2b by hydroxylamine restored 90% of the initial activity. The data showed that the incubation of PAP2b with DEPC plus phosphatidate can prevent the inhibitory effect of DEPC on enzyme activity. According to SDS- PAGE and gel filtration of PAP2b , this enzyme must have a hexamer structure and it can be concluded that one histidine residue is modified per enzyme subunit. The histidine residue in PAP1 plays a general base role and participates in dephosphorylation of phosphatidate. Our findings also revealed the role of histidine in the active site of PAP2b.This enzyme is likely to have a similar hydrolysis catalytic mechanism as its super family through a phosphohistidine intermediate.

p-190
EFFECT OF CODEINE ON SUCRASE ACTIVITY

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Sucrose is an important sugar in plants. It is also the main sugar in diets humans' l. Sucrase is a hydrolytic enzyme that breaks sucrose to its monomers; glucose and fructose. This enzyme has various isoforms in both plants and yeasts. The gastrointestinal system in human beings has also a kind of sucrase known as sucrase-isomaltase that resides in apical surface of the intestinal cells. Codeine is a common drug widely used in some countries as a pain reliever. The effect of codeine on the activity of the yeast sucrase was studied in this report. Non-competitive inhibition was observed using double reciprocal plots. The Km of enzyme did not change in the presence of different concentrations of codeine (0.5- 1.5 mM) and was determined to be about 11.5 mM. The Vmax of enzyme was estimated about 8.8 mM/min and it decreased in the presence of codeine. The Ki of codeine was measured by using the reaction rate and the initial concentration of the inhibitor according to the Dixon plot. The Ki was found to be 0.42 mM and the IC50 of codeine was determined to be about 0.875 mM.

p-191
EVIDENCE FOR THE ESSENTIAL ARGININE RESIDUE(S) IN THE CATALYTIC ACTIVITY OF GLUCOSE 6-PHOSPHATE DEHYDROGENASE FROM STREPTOMYCES AUREOFACIEN

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Glucose 6-phosphate dehyrogenase (G6PD) was purified from Streptomyces aureofaciens and inactivated with butandione. Incubation of the enzyme with butandione resulted in a rapid activity loss (80%) within 5 min, followed by a slow phase using a molar ratio to enzyme concentration of 100. Fluorescence studies showed a conformational change in the butandione-modified enzyme. NAD+ , NADP+ and glucose 6-phosphate protected the enzyme against inactivation. The data suggest that essential arginine residue(s) may be involved in the catalytic center of Streptomyces aureofaciens G6PD.

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PHOSPHOLIPASE A2 ACTIVITY OF VENOM AND FRACTIONS ISOLATED FROM IRANIAN VIPERA LEBETINA VENOM

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Objective: Snake venoms are complex mixtures containing many different biologically active proteins and peptides. These proteins are very specific for their molecular targets and stable in vitro and in vivo. Phospholipase A2 (PLA2) is an enzyme which hydrolyses phospholipids at sn-2 position, releasing fatty acids and lysophospholipids. Some venom contains a number of PLA2 isoforms. PLA2s are major components in snake venom. They display a wide range of biological effects including neurotoxic, myotoxic, cytotoxic, edema producing and anti-tumor effects. Vipera lebetina is one of the most poisonous snakes in Iran. Our studies on the crude venom have demonstrated the existence of PLA2 activity in the suspension of egg yolk substrate. Phospholipase A2 acted on lipoproteins in egg yolk and produced lysolecthin. The lysolecthin produced is able to solubilize the egg yolk suspension. Methods: The venom of Vipera lebetina was separated into five fractions, using gel filtration chromatography on Sephadex G-100 equilibrated with 20mM ammonium acetate buffer , pH= 6.8.These fractions were tested for PLA2 activity. Results & Conclusion: This activity was detected in PII and PIII. It was more prominent in PII.The specific activity of PI was about 2 times higher than that of crude venom.

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PURIFICATION OF BARLEY OXALATE OXIDASE USING ION EXCHANGE CHROMATOGRAPHY AND GEL FILTRATION

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Introdaction: Barley oxalate oxidase (OXO) is an apoplastic glycoprotein in biotic and abiotic stressed plants that is used widely in agricultural and food industries and medical diagnosis of kidney stones and oxaluria. In this study oxalate oxidase was purified from barley. Materials & Methods: Barley roots cultured in hydroponic stat were extracted in presence of sodium meta bisulphite. Oxalate oxidase was purified with a modified procedure including: ammonium sulphate fractionation, thermal treatment (60˚C, 10 min), and two step chromatography (Ion exchange and gel filtration). Purity and molecular mass of the product was estimated using SDS-PAGE .The enzyme activity was measured by a spectrophotometric method. Isoelectric points (pIs) of the separated enzymes were estimated using chromatofocusing. Results: Comparison of results showed that oxalate oxidase in this study was purified with higher recovery and solubility. The molecular mass of the enzyme was estimated to be 25-26 and 117-120 KDa, respectively in reducing and non-reducing SDS-PAGE. At least two isoenzymes with pIs of 6.8 and 6.1 were identified. Conclusion: According to data from this study, we can use the purification method of this enzyme for measuring oxalate in biological samples.

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COMPARATIVE EFFECTS OF L-AMINO ACIDS ON SERUM HIGH AND LOW MOLECULAR WEIGHT ALKALINE PHOSPHATASES

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Alkaline phosphatase (ALP) is an intrinsic plasma membrane enzyme found in many tissues . High molecular weight alkaline phosphatase might be considered as a tumor marker in prognosis of hepatocellular proliferative processes. Organ-specific inhibitors, which preferentially inhibit ALP of a specific organ, can aid in the differential analysis of ALP isoenzymes. The effects of L-amino acids on serum high and low molecular weight ALP was the major aim of this study. Using column chromatography on Sephacryl –S-300 and Agarose gel electrophoresis , high molecular weight alkaline phosphatase was separated from other ALP isoenzymes in sera of patients suffering from liver cancer. Data in this study showed that 10 mmol of L-Phe , L-Lue , L-Arg , L-Ser , L-Glu inhibited high molecular weight ALP by 30, 66 , 90 , 40 , and 6 percent and low molecular weight ALP by 30 , 66 , 64 , 4 , and 26 percent,respectively . According to our investigations L-Arg and L-Ser inhibit high molecular weight ALP more than low molecular weight ALP and L-Glu inhibits low molecular weight ALP more than high molecular weight ALP. These inhibitors can aid in the differential analysis of high and low molecular weight ALP.

p-195
INHIBITORY EFFECT OF SCOPOLAMINE (HYOSCINE) ON SUCRASE ACTIVITY

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Scopolamine (Hyoscine) is an alkaloid drug obtained from plants of the nightshade family (Solanaceae). It is an anticholinergic agent which acts as a competitive inhibitor at postganglionic muscarinic receptor sites of the parasympathetic nervous system. Scopolamine is indicated in adults for prevention of nausea and vomiting associated with motion sickness and recovery from anesthesia and surgery. Sucrase is found in the intestinal tract of human beings which breaks the sucrose into glucose and fructose. This enzyme is also found in plants and yeasts. In this study the effect of scopolamine on the activity of the yeast sucrase was studied. Double reciprocal plot determined a non-competitive inhibition in the presence of different concentrations of scopolamine (0- 3.6 mM). The Km was determined to be about 11.5 mM which did not change in the presence of scopolamine. The Vmax was about 8.5 mM/min and decreased upon increasing scopolamine concentration. The Ki of scopolamine was about 1.7 mM, measured using the reaction rate and the initial concentration of the inhibitor according to the Dixon plot. The IC50 of scopolamine was determined to be about 0.45 mM.

p-196
SEPARATION AND PARTIAL PURIFICATION OF HALOPHILE AMYLASE FROM THE MODERATE HALOPHILIC BACTERIA

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α-Amylases are universally distributed throughout the animal, plant and microbial kingdoms. Over the past few decades, considerable research has been undertaken to produce extracellular α-amylase by a wide variety of microorganisms. The major advantage of using microorganisms for the production of amylases is the economical bulk production capacity and easy manipulation to obtain desired characteristics. In the present report, we investigate the amylolytic activities of a newly isolated moderately halophilic bacterium (Halobacillus karajensis, Strain MA-2) and describe purification and some biochemical properties of the α-Amylase. This strain produced an extracellular amylase when cultivated in media containing 5 to 10% NaCl. The enzyme was purified from the liquid culture media by centrifugation, dialysis, ion exchange chromatography on Q-Sepharose and gel filtration on Sephacryl S-200. Molecular weight was estimated to be 60 KD by sodium dodecyl sulfate-gel electrophoresis. The enzyme showed a maximum activity at 0.5M NaCl . Optimum pH and temperature of amylase were found to be 7 and 60°C, respectively. The enzyme activity was enhanced by Ca^{2+}, Mg^{2+}, Ni^{2+}, and diminished by EDTA and inhibited by Zn^{2+}. These results may suggest that the isolated enzyme has the potency to be applied in industrial mass production.

p-197
STUDY THE INHIBITORY EFFECT OF ROSE BENGAL ON INFLAMMATORY PATHWAYS IN LPS-ACTIVATED MACROPHAGES

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Inflammation is a complex process related to diseases such as myocardial infarction, rheumatoid arthritis, Parkinson disease and multiple sclerosis. Inducible nitric oxide synthase (iNOS), cyclooxygenase II (COX-II) expression and the release of their products [nitric oxide (NO) and prostaglandin E2 (PGE2)] by inflammatory macrophages have critical roles in inflammatory conditions. Anti-inflammatory drugs have unpleasant side effects and sometimes are not effective. So, scientists try to find more effective compounds. Rose Bengal (RB) is water soluble xanthine dyes that have been used in medicine as a safe compound. In this study, anti-inflammatory effect of Rose bangal was investigated in inflammatory macrophages in vitro. J774A.1 macrophages (125×103 cell/well in 96-well plate) were treated with bacterial lipopolysachharide (stimulator for iNOS and COX-II and production of pro-inflammatory cytokines) and different concentrations of Rose Bengal (10-200 micromolar) and incubated at 37ºC. After 18 hours, NO production (by Griess method) and PGE2, TNF-α and IL-1 (By ELISA) were measured. Western blotting method was used for quantitative measurement of iNOS and COX-II expression. Results were analyzed with SPSS 11.0 (ANOVA). Different concentrations

of Rose Bengal caused a significant decrease ($p<0.001$) in the production of NO, PGE2 and pro-inflammatory cytokines in a dose-dependent manner. This decrease was associated with a decrease in iNOS and COX-II expression. The results showed a significant anti-inflammatory effect of Rose bangal and demonstrated the mechanism of its action. Therefore, Rose bengal may be an effective and safe anti-inflammatory compound and could be a good candidate for the treatment of inflammatory diseases in vivo.

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SESQUITERPENE DERIVATIVES ISOLATED FROM DOREMA KOPETDAGHENSE INHIBIT NITRIC OXIDE PRODUCTION IN LPS-ACTIVATED MACROPHAGES

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In Iran, the genus Dorema (Apiaceae) has been used as traditional medicines. Macrophages play major roles in the immunity and inflammatory responses. Oonce activated with bacterial lipopolysaccharides (LPS), they initiate the production of pro-inflammatory mediators such as nitric oxide (NO). The excessive synthesis of NO by inducible nitric oxide synthase (iNOS) plays a central role in inflammatory reactions. We recently discovered that dichloromethane extract of D.kopetdaghense inhibited NO production. Therefore, the analysis of this plant was undertaken to identify the active compounds. Through bioactivity-guided fractionations, two new sesquiterpene derivatives (kopetdaghins A and C) were isolated from aerial parts and kopetdaghins E was isolated from the roots of D.kopetdaghense. Their structures were elucidated based on HMBC and high-resolution MS. Murine macrophage-like cells (J774A.1) were activated with LPS (1μg/ml) with/without different concentrations (5-100 μg/ml) of these compounds. Their cytotoxic effects were measured using MTT assay. Griess reagent was used for measurement of NO production. Cytoplasmic extracts were prepared using cell lysis buffer and subjected to 8%SDS-PAGE. Western blotting was performed by transferring proteins to a PVDF membrane. The membrane was blocked with 2%BSA and incubated with anti-mouse iNOS antibody and then with secondary conjugated antibody. Subsequently, blots were developed using ECL-detection reagents and exposed to X-ray film. The protein bands of iNOS were scanned and densitometrically analysed by Imaging Densitometer. Purified novel sesquiterpene derivatives did not demonstrate any significant cytotoxicity but inhibited NO production and iNOS expression significantly in a dose-dependent manner in LPS-activated macrophages. So, they could be good candidates as safe anti-inflammatory agents.

p-199
OXIDATION AND S-NITROSYLATION OF HUMAN CYTOSOLIC THIOREDOXIN: A POSSIBLE ROLE IN CELL SIGNALING

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Reactive oxygen and nitrogen species are small molecules, implicated in cell signaling. The protein-disulfide reductase activity of the thioredoxin (Trx) system, containing Trx, thioredoxin reductase (TrxR) and NADPH can be inhibited by incubation with S-nitrosoglutathione (GSNO). We hypothesized this effect could result from S-nitrosylation of human Trx1 which besides Cys32 and Cys35 in its active site has three structural Cys residues in positions 62, 69 and 73. The number of nitrosothiols in fully reduced Trx1 treated with GSNO was calculated spectrophotometrically using a molar extinction coefficient of 920 M-1 cm-1 at 335 nm, showing 2.08±0.19 nitrosothiols. Different Cys-to-Ser mutants and wild-type Trx1 were nitrosylated to find the localization of nitrosothiols. S-nitrosylation of Cys69 and Cys73 was observed, and Cys32 and Cys35 were oxidized by the formation of a disulfide bridge. The protein-disulfide reductase activity of Trx was inhibited after nitrosylation of two Cys69 and Cy73; however, this effect was reversible and Trx regained its activity after a lag phase. H2O2 also inhibited Trx activity which was regained after a lag phase; however a different mechanism was involved that is the formation of a second disulfide between Cys62 and Cys69. Interestingly, Cys73 was not affected by H2O2, and no dimerization or oligomerization was observed. Mammalian cytosolic TrxR was not affected by either GSNO or H2O2. The transient inhibition of human Trx1 activity by H2O2 and NO probably plays a role in cell signaling via providing time for the transmission of oxidative and/or nitrosative signals like reversible inhibition of phosphotyrosine phosphatases.

p-200
KINETIC PROPERTIES OF o- DIANISIDINE PEROXIDASE IN PISTACHIO NUT EXTRACT

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Pistachio nut, produced by pistachio tree (pistacia vera L.), have been cultivated in Iran and consumed in some parts of the world since antiquity and are now considered worldwide as prime edible nuts. They are low in saturated fat, cholesterol free, and they are a rich source of essential nutrients, fiber and protein. There is, however, no information on their biochemistry. Peroxidases, hemoproteins that catalyze the H2O2-mediated oxidation of a broad range of substrates, are found abundantly in plants as numerous isoenzymes. In this work, o- dianisidine peroxidase activity was assayed in pistachio nut extract. The activity was measured by monitoring the H2O2- mediated oxidation of o-dianisidine at 460 nm. pH activity profile showed a single peak at 5.0. At optimum pH, Km, Vmax and catalytic efficiency were, respectively, 315 μM, 140 μM/min and 0.44/min with o-dianisidine as the varied substrate, and 232 μM, 95.2 μM/min and 0.41/min with H2O2 as the varied substrate. Vmax and

catalytic efficiency was expressed per mg protein extract. IC50 was 2.5 μM for KCN and 72 μM for NaN3. When o-dianisidine was the varied substrate, inhibition was noncompetitive by KCN and competitive by NaN3; but when H2O2 was the varied substrate, inhibition was competitive by KCN and noncompetitive by NaN3. Activity dropped by 30% after incubation of the extract for 10 min at 30-60 °C, and dropped after incubation at higher temperatures. Data suggested the presence of at least one o- dianisidine peroxidase isoenzyme in pistachio nut extract.

p-201
KINETIC PROPERTIES OF FLAVOCYTOCHROME B2 IN SATUREJA HORTENSIS L. LEAVES EXTRACT

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Satureja hortensis L. (summer savory) is widely cultivated in Iran and is also well known in Iranian traditional medicine as a remedy for various ailments. Flavocytochrome b2 (L- (+)-lactate ferricytochrome c oxidoreductase) (EC 1.1.2.3) is a tetramer, in which each protomer contains both FMN and protoheme IX. It takes part in a catalytic reaction that transfers electrons from lactate to cytochrome c and then to the terminal respiratory chain, or from lactate to other electron acceptors such as potassium ferricyanide, as follows: lactate + 2 ferricytochrome c → pyruvate + 2 ferrocytochrome c. In this work, the enzyme activity was investigated in S. hortensis leaves extract. Flavocytochrome b2 activity was measured by following spectrophotometrically, at 420 nm, the reduction of ferricyanide at pH 9.5, in a 0.1 M citrate-phosphate-borate buffer.The pH activity profile, using potassium ferricyanide as the electron acceptor, showed one peak at pH 9.5 and a shoulder at pH 8. Kinetics parameters were determined at optimum pH, giving the following results: Km and Vmax were, respectively, 6.1 mM and 63.65 μM.min-1 with lactate as the varying substrate, and 37 μM and 42.3 μM. min-1, with ferricyanide as the varying sustrate. At lactate concentrations over 100 mM, substrate inhibition was observed. At pH 9.5, the enzyme showed maximum activity after preincubation at 30˚C. Malate was determined as competitive inhibitor for flavocytochrome b2 at pH 9.5 and IC50 was 28 mM. Results showed the presence of at least one isoenzyme of flavocytochrome b2 in S. hortensis leaves extract.

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PURIFICATION AND PROPERTIES OF PEROXIDASE FROM THE ROOT OF TURNIP

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Peroxidase (EC 1.11.1.7) is a heme containing enzyme that has been widely used as a reagent for organic synthesis and biotransformation as well as in coupled enzyme assays, chemiluminescent assays, immunoassays and the treatment of waste waters. Peroxidase activity in the field grown turnip roots (Brassica napus var. okapi) was measured during four stages of growth. The most activity was found in the ripening stage. Peroxidase of the root in this stage was partially purified by ammonium sulfate fractionation and DEAE-Sephadex A-50 chromatography. Two peaks were detected with high peroxidase activity (TP1 and TP2). Based on guaiacol assay, specific activity of TP1 and TP2 were 67.94 (u/mg) and 1.14 (u/mg), respectively. The specific activity in TP1 was 10.47 fold its value in crude extract. Km of TP1 for H2O2 was 5.5×10^{-4} M and its Vmax was 5×10^{-3} MS-1. TP1 was most active at pH 6 and 50 °C. pH stability and thermostability of this enzyme was determined. The results show that after 30 min incubation of the enzyme at 70 °C, its activity was reduced to 45% of its activity at 25 °C. NaN3 and NaCN have inhibitory effects on TP1 activity. A 50% reduction in TP1 activity is observed by 0.299 mM of NaN3 or 0.144 mM of NaCN.

p-203
PREPARATION AND CHARACTERIZATION OF IMMOBILIZED ACETYLCHOLINESTERASE

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In this study adsorptive immobilization of acetylcholinesterase (AChE) from Torpedo california on functionalized mesoporous hydrophobic matrix: octadecyl substituted porous silica (at 220 μmol/g matrix) are presented. Immobilization product can be constituted as a sensory element to construct enzyme-based flow-biosensors. Efficient immobilization was achieved after simple adsorption of the enzyme on the carrier. In spite of the low quantified dissociation constant at the 10^{-6}, preparation was characterized with high operational stability at aqueous conditions up to 40 days. Moreover immobilization process led to a change in the pattern of activity versus adsorbed protein, most probably due to protein-protein interaction in the adsorption product.

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SUPEROXIDE DISMUTASE ACTIVITY IN CROCUS SATIVUS L CORMS EXPOSED TO CADMIUM

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Cadmium (Cd2+) is a widespread environmental pollutant with high toxicity to humans, animals and plants. Among other effects, it produces oxidative stress possibly by generating free radicals and reactive oxygen species (ROS). Crocus sativus L., the plant producing the renowned saffron spice, is propagated only via its corms. These would be most directly exposed to Cd2+ in case of soil or water contamination by the metal. Plants defense system against oxidative stress includes superoxide dismutase (SOD), an enzyme considered as the first line of defense against ROS-caused injury. In this study, SOD activity was investigated in

Crocus sativus L. corms cultivated for 3, 6 and 9 days in the presence of various Cd2+ concentrations, and compared to that in dormant corms. The enzymatic activity was measured spectrophotometrically by a method based on pyrogallol oxidation. Results showed that in the absence of added Cd2+, SOD activity in corm extract doubled within 6 days of cultivation and remained at that level after 9 days. Results were similar when up to 2 mg/l Cd2+ was added. After 3 days cultivation with 5 mg/l Cd2+, SOD activity reached 1.5 times that in dormant corms; it remained at that level until day 9. For corms cultivated in higher Cd2+ concentrations (20-50 mg/l), SOD activity was 1.5 times that in dormant corms at days 3 and 9, but was reduced by 50% at day 6. Results showed that Crocus sativus L. corms SOD activity was unaffected by low Cd2+ concentrations but inhibited by higher Cd2+ concentrations.

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KINETIC STUDY OF 7 PLANT EXTRACTS EFFECTIVE AGAINST ALPHA GLUCOSIDASE ENZYME (A TARGET ENZYME FOR HYPERGLYCEMIC PATIENTS THERAPY)

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Enzyme inhibitors play a significant role in preservation of human health. Glycosidases located in the brush-border surface membrane of intestinal cells are the key enzymes of carbohydrate digestion. Carbohydrates have to be broken down enzymatically in the intestine to monosaccharides before their absorption. The other cellular glycosidases are vital for the processing of glycoproteins and glycolipids which are involved in various biological reactions such as immune responses, metastasis of cancer, and viral infections. No doubt, glycosidase inhibitors would be the most powerful tools for influencing the kinetics of intestinal carbohydrate digestion with immediate effects on glucose absorption, blood sugar increase and insulin response. Damask rose, Origanum, Pistachio, Myrtle, Gallnut, Mint and Green tea extracts are strong inhibitor of alpha glucosidase as observed in our previous study. Methanolic or aqueous extracts prepared by maceration method. Enzyme assay of α-glucosidase was observed spectrophotometrically at pH 6.8 and 37°C using p-nitrophenyl-α-D-glucopyranoside as a substrate. In order to examine the inhibition type produced by these plants extracts, alpha-glucosidase activity was measured under optimum conditions with increasing concentrations of PNP-glycoside (0.25–1 mM) in the absence or presence of the extracts. Inhibition type for these extracts was determined by Lineweaver–Burk plot analysis. Our results demonstrated noncompetitive inhibition pattern for alpha-glucosidase activity by all of these extracts. The Km value was 1.4 mM ,Vmax was0.04 µmol/min and Vmax in presence of 0.25µg of Damask rose, Origanum, Pistachio, Myrtle ,Gallnut Mint and Green tea extracts were 0.011 ,0.025 ,0.022 ,0.009,0.009 ,0.0.021 and 0.016 µmol/min, respectively. Further studies are under investigation To elucidate the in vivo effectiveness of these plants.

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INHIBITION OF HUMAN CERULOPLASMIN BY CADMIUM

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The inhibition of human ceruloplasmin (Cp) by cadmium was studied in vitro. Cadmium (Cd), an extremely toxic metal and nonessential trace element in humans, inhibited ceruloplasmin progressively up to a concentration of 2 mM when about 75% of the enzyme activity was lost. Loss of activity was reversible. The inhibition was noncompetitive with respect to phenylene diammonium dichloride (PPD) with a Ki value of about 1.1 mM calculated from the slope replot. Cadmium also increased maximum emission spectrum of the intrinsic protein fluorescence. Sulfhydryl compounds such as glutathione (1.2, and 12mM), β-mercaptoethanol (12 mM) or dithiothreitol (12 mM) protected the enzyme activity against cadmium inhibition and restored the native protein fluorescence. The data suggest that sulfhydryl groups are involved in cadmium inhibition. Cadmium, probably inhibits human ceruloplasmin through binding to sulfphydryl groups on the enzyme and inducing a conformational change in the enzyme which results in a decrease of catalytic activity.

p-207
A SIMPLE METHOD FOR DETERMINATION OF ALKALINE PHOSPHATASE ISOENZYMES IN SERUM

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The enzyme alkaline phosphatase (EC3.1.3.1, ALP) is a zinc-containing glycoprotein catalyzing the hydrolysis of phosphomonoesters at alkaline pH. ALP is synthesized in a variety of tissues and has different isoenzymes and isoforms. Four variants of ALP have been identified in domestic animals: bone ALP (B-ALP), intestinal ALP, liver ALP (L-ALP), and, in dog, corticosteroid-induced ALP (C-ALP). Efforts have been made to develop analytical techniques to differentiate different isoezymes of ALP in blood serum. The purpose of this study was to use a chromatographic method to separately measure bone, liver and steroid-induced ALP in serum of healthy and diseased dogs. Serum samples were collected from 5 clinically healthy and 4 femur- fractured Iranian sheepdogs. Tissue samples were obtained by autopsy of dogs referred to veterinary clinic after road car accident. 5.0ml of serum or tissue extract was loaded onto a DEAE-Sephadex column equilibrated with 0.01 M Tris-HCl buffer pH 7.2. Proteins were eluted with a linear 0.0-0.4M NaCl gradient. ALP activity was measured by a kinetic method using para-nitrophenyl phosphate as substrate. Results indicated resolution of ALP activity in 2 major peaks corresponding to the B-ALP and L-ALP and a minor peak related to C-ALP, in the order of elution form the column. The peak related to B-ALP isoenzyme was elevated in the serum of femur-fractured dogs. This peak returned to normal after healing. The peaks corresponding to C-ALP was elevated in dexamethasone-treated dogs. Taken together, the results of this study shows that this simple procedure can be used to differentiate different isoenzymes of ALP and provides a fast and inexpensive diagnostic approach to diseases of dogs.

Application of this method to human serum samples is under investigation.

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IMPROVING BIOCHEMICAL PROPERTIES OF BETA-GLUCAN THROUGH CONJUGATION WITH LYSOZYME

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Beta-glucan is a polysaccharide in the form of fiber found in different sources such as cereal grains, yeasts and mushrooms. As a functional and nutritious ingredient, it has blood sugar reducing, weight reducing, immune modulatory, lipid lowering and anti-carcinogenic effects. Nevertheless low solubility of this compound has limited its application. The purpose of this study was to attach barley beta-glucan to lysozyme through Maillard-based reaction. The extent of conjugation was followed by sugar analysis, SDS-PAGE and gel filtration chromatography. The results of electrophoresis indicated diffused bands which is an indicative of diverse products with different molecular weights. Glycosylation of lysozyme with a 1:1 weight ratio of protein to beta-glucan, held at 60 ºC for one week under a relative humidity of 79%, resulted in coupling of 0.014 mol beta-glucan to one mol lysozyme. The modified polysaccharide showed a better solubility at different pHs (3, 7 and 9) and temperatures (25, 40 and 60 ºC), also higher emulsion stability, foam capacity and heat stability in comparison with unmodified polysaccharides. The results of this study indicated that the beta-glucan-lysozyme conjugate can be considered as a new functional ingredient in different food and drug systems with the potential of antimicrobial properties due to the lysozyme moiety.

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EFFECT OF DEXTRAN SULFATE CONJUGATION ON PHYSICOCHEMICAL AND BIOLOGICAL PROPERTIES OF LYSOZYME

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The natural and non-toxic protein–polysaccharide conjugates can be expected to have significant potential for use in the food and health industries. The use of lysozyme and dextran sulfate in pharmaceutical and health care products has been quite broad in scope and application. Furthermore, lysozyme has widespread application as a natural antimicrobial or preservative agent in food industries such as dairy industry. It is desirable to extend application spectra of these polymers with the change of their physicochemical and biological properties by non-toxic chemical modifications. In this study, attachment of dextran sulfate (5KD) to lysozyme was performed by Maillard-based reaction and the effect of modification on physicochemical properties (solubility, thermal stability and emulsifying properties) and antimicrobial activity of lysozyme was studied. Reaction mixtures containing lysozyme and dextran sulfate (1:5 weight ratio) were dry-heated at 60˚C and 79% relative humidity for 1 week. SDS-PAGE analysis, sugar analysis and cation exchange chromatography confirmed the covalent attachment of the polysaccharide to the protein. Approximately 1.1 moles dextran sulfate was covalently linked to 1 mole lysozyme. The resulting conjugate had better emulsifying activity and stability than dextran sulfate and lysozyme alone and glycated proteins exhibited a better thermal stability and solubility (in pH 7, and 9 and temperatures of 25, 40, and 60˚C) as compared to heated lysozyme. Furthermore lysozyme-dextran sulfate conjugate revealed significant antibacterial activity against both Gram-negative and Gram-positive bacteria probably due to excellent emulsifying properties in comparison to lysozyme and dextran sulfate alone.

p-210
DENATURATION STUDIES ON THE PEPSIN BY SODIUM DODECYL SUIPHATE

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Pepsin (E.C.3.4.23.1) is an aspartic proteinase of the gastric juice.. It consists of a single polypeptide chain of molecular weight 34.64 kDa and 327 amino acids. In the present study, the stability of pepsin has been investigated by spectrophlorimetric techniques in the presence and absence of sodium dodecyl sulphate (SDS). It has been observed that (1) By increasing pH, enzyme stability was increased (2) At pH=2 and 5, the stability of pepsin was increased in the presence of SDS (3) In the presence of SDS and pH=2. 5, the flourescent intensity and λMAX decreased.

p-211
TRANSITION METAL-INDUCED STIMULATION OF LIGNIN PEROXIDASE ACTIVITY IN CROCUS SATIVUS L. CORMS

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Peroxidases are hemoproteins that fulfill a wide range of physiological functions while scavenging hydrogen peroxide produced by oxidative stress or as by-products of aerobic metabolism. Exposure to transition metals is one of the causes of oxidative stress, and increasing environmental contamination by transition metals has been the cause of growing concern. Plants are among the organisms that would be primarily affected by metallic contamination in soil and water. Hhopefully, they are equipped with an elaborate anti-oxidative stress defense system that includes numerous peroxidase isoenzymes. Lignin peroxidases (LPOX), for example, plays an important role in lignin degradation as well as in the strengthening of cell wall, while scavenging H2O2. In this work, LPOX activity was assayed in Crocus sativus L. corms cultivated in the presence of increasing cadmium (Cd^{2+})

concentrations for up to 9 days. LPOX activity was measured by monitoring the H2O2-mediated disappearance of ferulic acid at 310 nm. Ferulic acid is a model substrate for studying LPOX-catalyzed polymerization of phenolic compounds in vitro. Assays were conducted at days 0 (dormant corm), 3, 6 and 9 of cultivation. Results showed that after 9 days of cultivation without added Cd^{2+}, LPOX activity increased 3 times of the value found in dormant corms. In 0.1-5 mg/l Cd2+, the activity reached 4 times that of the dormant corms after 9 days of cultivation while in 20-50 mg/l Cd^{2+}, LPOX activity was 6 times that of dormant corms. Thus Cd^{2+} stimulated LPOX activity in Crocus sativus L. corms possibly limits Cd^{2+} toxicity by increasing lignification.

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KINETIC PROPERTIES OF GUAIACOL PEROXIDASE IN CROCUS SATIVUS L. ROOTS

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Peroxidases (EC 1.11.1.7) are hemoproteins that catalyze the oxidative transformation of organic reactants with H2O2 as the oxidant. Plant peroxidases fulfill a variety of functions such as lignification, cross-linking of cell wall proteins, etc., while scavenging H2O2. peroxidases that utilize guaiacol (o-methoxyphenol) as reducing substrate in vitro are referred to as guaiacol peroxidases. In this work, guaiacol peroxidase activity was assayed in Crocus sativus L. roots.Crocus sativus L. is the plant producing saffron, the most expensive spice in the world, which has been used since antiquity for culinary and medicinal purposes, and as a dye. The peroxidase activity was measured in Crocus sativus L. roots extract by following spectrophotometrically the H2O2-mediated oxidation of guaiacol at 470 nm. The pH activity profile exhibited a peak at 4.0 and a shoulder at 6.5. At pH 4.0, Km and Vmax were, respectively, 5.36 mM and 0.45 mM/min.mg prot with guaiacol as the varied substrate, and 2.13 mM and 0.42 mM/min.mg prot. with H2O2 as the varied substrate. Thermal stability of the enzymatic activity was investigated by incubating Crocus sativus L. roots extract at various temperatures for 10 min, then prompt cooling in ice and assay at room temperature (~ 25ºC). Enzymatic activity was maximum after incubation at 35ºC and then decreased progressively and reached zero as temperature increased to 70ºC. NaN3 and KCN inhibited the enzymatic activity with IC50 of, respectively, 0.035 mM and 0.015 mM. Data suggested the presence of at least one guaiacol peroxidase isoenzyme in Crocus sativus L. roots.

p-213
STUDY OF G6PD DEFICIENCY IN A POPULATION REFERRED TO ABOOZAR HOSPITAL OF AHWAZ.

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Introduction: Glucose 6 phosphate dehydrogenase (G6PD) deficiency is one of the most common inherited disorders, with approximately 400 million people affected world wide. More than 140 different mutations of the G6pD gene have been identified. G6PD deficiency is unrecognized in most affected individuals. However, it may have a clinical expression, such as acute hemolytic anemia, favism and neonatal hyperbilirubinemia. Different variants of the enzyme are found in African, Mediterranean, and Asian populations. The aim of this study was to determine the prevalence of G6PD deficiency in a population referred to Aboozar hospital of Ahwaz Materials and methods : In the present study, the blood samples were collected from 200 subjects (Newborns 100, children 100). Diagnosis of severe G6PD deficiency was done by fluorescent spot test. Fluorescence readings were performed at the beginning and 5, 10, and 20 minutes after incubation, and were classified in to three groups: bright fluorescence, weak fluorescence, and no fluorescence. Results: The range of hemoglobins in the population was 3.8 – 13 g/dl in children and 9.2- 17.8 g/dl in newborns.. Of the 200 tested blood samples, 50 (25%) samples showed weak fluorescence or no fluorescence spots. The prevalence of G6PD deficiency was 14% in newborns and 36% in children. Conclusion: The high incidence of G6PD deficiency implies that this inherited metabolic disorder is a serious health problem in Ahwaz, and it is necessary to carry out large scale screening in the whole population in order to prevent G6PD deficiency related health problems.

p-214
EXPRESSION OF GLUTATHIONE S-TRANSFERASE IN HEPATOCYTES DERIVED FROM HUMAN BONE MARROW MESENCHYMAL STEM CELLS

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Glutathione S-transferases (GSTs; EC 2.5.1.18) are a family of isoenzymes which are widly distributed in animal tissues; their physiological function is detoxification of xenobiotics and drugs. Human hepatocytes as one of the important sources of GSTs have been extremely useful for the investigation of drug metabolism and toxicology. Today , the differentiation of human stem cells to hepatocytes has become routine in some of the laboratories, however the status of drug metabolism system in these cells is not fully understood In order to investigate the activity of GSTs in the differentiated hepatocytes, human bone marrow derived mesenchymal stem cells (MSCs) were first differentiated into hepatocytes in the presence of hepatocyte growth factor (HGF), dexamethasone and oncostatin M in vitro, then the differentiated cells were examined for their ability to express liver specific markers (albumin and α- fetoprotein), using reverse transcriptase polymerase chain reaction (RT-PCR) and immunocytochemistry . Moreover, GST activity was compared in differentiated cells, undifferentiated cells (MSCs) as negative control, and HepG2 cells as positive control, using 1-chloro-2, 4-dinitrobenzene (CDNB) as a substrate. The results show that the GST activity was not detectable in the MSCs , whereas GST enzyme activity was 0.35 and 0.03 µg /min /mg protien in differentiated cells and in HepG-2 cells, respectively .This data indicate that the MSCs derived human hepatocytes are active and with a potential capacity for detoxification.

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REGULATORY EFFECT OF THE SUBSTRATE ON THE ADENOSINE DEAMINASE ACTIVITY

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Adenosine deaminase (ADA), a purine catabolic enzyme, catalyses the deamination of adenosine and 2-deoxy adenosine to inosine and 2-deoxy inosine irreversibly. The enzyme activity and UV spectra were spectrophotometrically obtained in 50 mM Tris buffer, pH 7.5 at room temperature as well as physiological and pathological temperatures (25, 37 and 42 ^{o} C) in different concentrations of the substrate. The changes in secondary structures of ADA were examined by analyzing the CD spectra of the enzyme in the same buffer at 25, 35 and 45^{o}C. According to the results, ADA has more activity and different secondary structures at 37^{o} C compared with 25 and 42^{o} C. The Michaelis-Menten curves showed super-activation of ADA in certain concentrations of adenosine, implying the existence of a regulatory site for the substrate on ADA molecule. This assumption has been confirmed by docking analysis.

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THE INFLUENCE OF PUTRESCINE ON THE KINETICS AND THE STRUCTURE OF BOVINE PANCREATIC RIBONUCLEASE A

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Putrescine is a natural diamine with a net positive charge at physiological pH which is distributed in the eukaryotic and prokaryotic kingdom. In this study the influence of putrescine on the kinetic and structural behaviors of bovine pancreatic ribonuclease A was investigated using difference spectrophotometry, circular dichroism, binding studies and fluorescence spectroscopy. Our advanced kinetic analysis has shown that in the presence of putrescine there are two ligand binding sites on the enzyme structure. On the other hand, our binding studies in the presence of putrescine indicated that there is one specific binding site on the enzyme which is occupied by putrescine as a ligand. Therefore, it is logical to say that there are two RNase isoforms in the solution which can be detected kinetically but their behaviors are identical in the binding studies. The possibility for the existence of two isoforms of the enzyme is also proposed by some researchers using several biochemical and biophysical techniques. Structural properties of the enzyme (especially on the secondary level) in the presence of putrescinee indicated that some considerable alterations occurs in the enzyme structure (e.g. alpha-helices and beta-sheets) supporting our findings about the decrease of the enzyme stability in the presence of putrescine when the thermal variations are exerted on the enzyme. In conclusion, putrescine works as an inhibitor for RNase A and has distinct destabilization effect on the enzyme structure.

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SUICIDE INACTIVATION OF ENZYME DEVELOPS A MIRAGE IN DETERMINATION OF KM

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Exposure of an enzyme to its suicide substrate is concurrent with a continuous irreversible decrease in its catalytic activity. The "partition ratio" is a determinable "stoichiometry" between the diminished enzyme activity and consumed substrate during catalysis. Determination of Km of a given enzyme with a suicide substrate is faced with an experimental problem. Start point of determination of initial velocity, V_o, is lagged in relation to exact initiation of reactions. Therefore, diminised enzyme activity during lag time exerts some underestimation V_o which is expanded with an elevation of substrate concentration. This exerts a pseudo-curvature on the zero-order region of Michaelis-Menten kinetics, leading to a faulty estimation of Km. In the present study, catalase / hydrogen peroxide system was used as a desirable model to show how suicide inactivation can produce faultyl Km values when a traditional procedure is used. The extracted wrong Km (about 30 mM for this system) found to be over 20 times lower than the authentic ones. Also, it was shown that the determination of Km under the same physicochemical conditions but with different lag times can lead to incorrect and l diverse Km values. We were able to improve the estimation of Km of catalase by using a kinetic equation that offsets the effect of suicide inactivation on the determination of V_o.

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ALPHA-1-ANTIRYPSIN PHENOTYPES AND ACTIVITIY AND ITS CORRELATION WITH ANTINEUTROPHIL CYTOPLASMIC ANTIBODY IN IRANIAN PATIENTS WITH ALOPECIA AND PSORIASIS

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Backgrand/Objective: Alpha-1-antirypsin (AAT) is a natural inhibitor of proteinase3 (PR3), a neutrophil granular protein and major auto-antigen of antineutrophil cytoplasmic antibody (c-ANCA). AAT has been associated with a variety of conditions including emphysema, neonatal hepatitis, cutaneous and systemic vasculitis, rheumatoid arthritis, psoriasis and some other autoimmune diseases. Methods: In this study sera from thirty six Iranian patients with autoimmune disease (psoriasis, N=20 and Alopecia, N=16) were investigated to find out if there is a correlation between AAT phenotypes and activity with ANCA. ANCA was

detected in patients' sera using the standardized Indirect Immunofluorescence (IIF) test on ethanol-fixed granulocytes. The activity of the AAT as its potential inhibitory capacity on trypsin (TIC) was measured spectrophotometrically using N-benzoyl-DL-arginine-p-nitro anilide(BAPNA) as substrate at 400nm. The concentration of serum AAT was measured using nephelometric assay,. Phenotyping of the samples were carried out using Isoelectric focusing (IEF) with polyacrylamide gel containing ampholyte (pH=4.2-4.7). Results: 14% of patients with psoriasis were c-ANCA positive and all patients with alopecia were c-ANCA negative The mean of TIC in patients with psoriasis and alopecia were 4.532±1.12 and 2.23±0.9 µmol/ml/min, respectively. The concentrations of serum AAT were 233.14±29.2 and 118.13±19.1 mg/dl, respectively. Conclusion: We conclude that there is no significant difference between AAT activity and ANCA positive tests in psoriasis ($p>0.05$), but a significant difference exists between AAT activity and ANCA positive tests in alopecia ($p<0.01$). AAT phenotypes in all groups were MM, except for two samples of psoriasis patients that were MZ and two samples which were SS.

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A NOVEL ONE-STEP EXTRACTION METHOD FOR THE RECOVERY AND PURIFICATION OF RECOMBINANT PHENYLALANINE DEHYDROGENASE

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Phenylalanine dehydrogenase (PheDH; EC 1.4.1.20) is a member of amino acid dehydrogenase family that catalyzes the reversible NAD^+-dependent oxidative deamination of L-phenylalanine to phenylpyruvate. It has received much attention as a biocatalyst for phenylketonuria (PKU) in newborn screening tests and for the synthesis of chiral compounds as well. The main goal of this study was to find a simple and rapid alternative for purifying PheDH to replace the previously used methods. Aqueous two-phase systems (ATPS) composed of polyethylene glycol 6000 (PEG-6000) and ammonium sulfate were investigated for purification of recombinant Bacillus badius PheDH. The influences of system parameters including; PEG molecular weight and concentration, (NH4)2SO4 concentration, pH, temperature, phase volume ratio (VR), tie-line length (TLL) and NaCl salt addition on enzyme partitioning were studied. The purity of enzyme was also analyzed by SDS-PAGE electrophoresis. A single-step procedure was developed for the extraction and purification of recombinant PheDH from E.coli BL21 (DE3). The optimized system was 8.5% (w/w) PEG-6000, 17.5% (w/w) (NH4)2SO4 and 13% (w/w) NaCl at pH 8.0. The specific activity, yield, purification factor, recovery and partition coefficient were 10424.97 U/mg, 95.85%, 474.3, 141% and 92.57, respectively. It was found that the PEG molecular mass, pH, temperature and NaCl concentration had significant effects on enzyme partitioning. Also the Km values for L-phenylalanine and NAD+ in oxidative deamination were 0.21 and 0.13 mM, respectively. The data presented in this study demonstrated the potential of ATPS as a versatile and scaleable process for recovery and large-scale purification of recombinant PheDH.

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SCREENING, ISOLATION, PURIFICATION AND CHARACTERIZATION OF PULLULANASE FROM A THERMOPHILIC STRAIN OF AN HOT SPRING

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A thermophilic strain L14, isolated from Iranian hot springs, produced an extracellular pullulanase upon growth on optimized liquid medium. The strain is likely to be Geobacillus stearothermophilus since the analysis of 16S rDNA gene sequence showed highest similarity (99%) with this strain (accession no.). The enzyme was purified by ammonium sulfate precipitation and anion exchange chromatography. The purified enzyme showed a band on SDS-PAGE with an estimated molecular mass of 130 kDa for monomeric enzyme. The partially purified enzyme had an optimum pH of 5.5 and an optimum temperature of 65°C. It had good stability at 60-70°C. The enzyme could hydrolyze pullulan and starch. The Km value for the enzyme activity on pullulan was 0.044g% (w/v) and Km on soluble starch was 0.5g % (w/v). The products of enzymatic reaction on pullulan and starch were glucose, maltose and maltotriose. It has been suggested that the purified pullulanase from Geobacillus sp. L14 is classified under pullulan hydrolase type III. To our knowledge this Geobacillus pullulanase is the sole pullulan hydrolase type III known in Bacillus strains to date.

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AGGREGATION OF GLUTAMATE DEHYDROGENASE: ROLE OF ALLOSTERIC EFFECTORS

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Glutamate dehydrogenase (GDH) is found in nearly every organism and plays a pivotal role in nitrogen and carbon metabolism. The beef liver enzyme consists of six identical protomers and its activity is modulated by a number of small molecules, with ADP and GTP being the most investigated activator and inhibitor, respectively. It has been shown that allosteric effectors stabilize particular conformational states of the macromolecule. Activation by ADP has been found associated with an increase and inhibition by GTP, Zn, steroids and thyroxine with a decrease in stability toward thermal denaturation. Moreover the tendency of the hexameric macromolecule to polymerize under the influence of these molecules has been established. In this study we investigated the process of heat-induced aggregation of GDH, assessing the preventive effects of the regulatory molecules on this process. It was shown that inhibitors such as NADH and GTP increase

the rate of aggregation, while activators such as ADP and L-leucine provide total protection. Furthermore, the positive effectors can strongly preserve the native structure of the enzyme but the negative effectors cause destabilization. Tm of the enzyme was found to increase by ADP and diminish in the presence of NADH. CD and fluorescence spectroscopy have shown that heating in the presence of ADP results in minimal changes in the structure of the enzyme. In the presence of NADH, GTP or NADH + GTP, more extensive changes were observed.

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AQUEOUS TWO-PHASE PARTITIONING OF NAD+-DEPENDENT PROLINE DEHYDROGENASE FROM CITROBACTER FREUNDII

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NAD+-dependent proline dehydrogenase (ProDH, EC 1.5.99.8) catalyzes the reversible oxidative deamination of L-proline to 1-pyrroline 5-carboxylic acid. This enzyme plays an important role in synthesis of L-proline as a building block for production of herbicides, insecticides and pharmaceuticals. Partition behavior of ProDH in an aqueous two-phase system (ATPS) containing polyethylene glycol 4000 (PEG-4000) and magnesium sulfate was studied. The effects of PEG molecular weight and its concentration and also MgSO4.7H2O concentration on ProDH partitioning were investigated. The optimal system for enzyme partition was 16% (w/w) PEG and 10.5% (w/w) MgSO4.7H2O under pH 7.0. The yield, purification factor, recovery and partition coefficient were 70.6%, 1.90, 73% and 0.84, respectively. In conclusion, our obtained data suggested that this ATPS can be used as a rapid and effective technique for extraction and purification of ProDH.

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PURIFICATION AND PROPERTIES OF GLUTAMINE SYNTHETASE FROM ESCHERICHIA COLI

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Glutamine synthetase (EC 6.3.1.2) purified over 120 fold from Escherichia coli, formed a single band on sodium dodesyl sulfate-poly acrylamide gel with a subunit molecular weight of 40 KDa .The native enzyme (260 KDa) was composed of six identhcal subunits .The purification procedure included: precipitation with (NH4)2SO4 , ion exchange chromatography on cellulose DE-32 and CM-32, gel filteration on Sepharose CL-6B and chromatography on AH-Sepharose 4B. The enzyme had a specific activity of 140 u/mg protein when assayed by measuring the rate of the formation of glutamyl hydroxamate using hydroxylamine as a substrate .The PH optimum of the enzyme reaction was 7.2 . The purified enzyme was activated by 10% ethylene glycol .The enzyme was inhibited by physiological concentrations of urea.

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EFFECT OF TEMPERATURE ON POLYPHENOL OXIDASE ACTIVITY IN DORMANT CROCUS SATIVUS L. CORMS

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Polyphenol oxidase (PPO) (monophenol, o-diphenol: O2 oxidoreductase, EC.1.14.18.1, EC1.10.3.2) catalyzes the hydroxylation of monophenols to o-diphenols (monophenolase activity) and the oxidation of o-diphenols to o-quinones (diphenolase activity), leading to browning in plants. Crocus sativus L., cultivated since ancient times as the source of saffron, is a triploid plant that can be propagated only via its corms which undergo a period of dormancy. Understanding the processes taking place in the corm is essential to preserve the plant and improve its quality. In a previous study, both monophenolase and diphenolase activities were detected in dormant Crocus sativus L. corms. In this study, the effect of temperature on both activities was investigated. Dormant Crocus sativus L. corm extract was incubated for 10, 30 and 60 minutes at various temperatures and, after brief cooling in ice, was tested for either mono- or di-phenolase activity. Results showed that both activities were optimum after incubation at 35°C; however, the monophenolase activity increased to 160% after 60 min at 35°C while the diphenolase activity increased to 260% after 30 min at 35°C. Monophenolase inactivation was observed after 30 min at 80°C although some residual activity (30%) was still detectable after 10 min at 80°C. Diphenolase inactivation was observed after 30 min at 60°C, 10% of activity being still detectable after 10 min at 60°C. Arrhenius plots showed transition temperature of , 60°C and 55°C for mono- and di-phenolase activities,respectively. Results reveal that, with respect to temperature, the monophenolase activity exhibits a greater stability than the diphoneolase activity.

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COMPARATIVE EFFECT OF GABAPENTIN AND LAMOTRIGINE ON LIVER ENZYMES AND WEIGHT OF MALE RATS

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Objective: Gabapentin (GPN) and Lamotrigine (LTG) are two new antiepileptic drugs used in the control of partial and secondary seizures. This study was undertaken to find out the effect of these drugs on liver enzymes and body weight of rats.

Material s and Method: Adult male Wistar rats, weighting 180-220 g were divided into 4 experimental and one control groups. The control group received normal saline. Groups 1 & 2 received therapeutic and toxic doses of GPN respectively, and groups 3 & 4 received therapeutic and toxic doses of LTG. The experimental period was 60 days. After this period, animals were weighed and LFT tests were carried out with autoanalyser. Results: Statistical analysis showed that in groups 2 , 3 and 4, the serum levels of AST, ALP, ALT, direct bilirubin and total bilirubin, increased significantly and in groups 2 and 4, total protein and Alb decreased significantly, but the changes of these parameters in the groups receiving therapeutic doses of GPN was not significant compared with the control group. The therapeutic doses of LTG & GPN do not interfere with body growth rate, but toxic doses of LTG may cause reduction and high doses of GPN may enhance body growth rate. Conclusion: It appears that the effects of GPN on liver function and hepatocellular damage are very mild as compared with LTG. The effect of these drugs on weight may be due to their effects on appatite.

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PURIFICATION AND CHARACTERIZATION OF A NEW α-AMYLASE FROM AN ACID-NEUTRALIZING BACTERIUM, BACILLUS SP.GUF8

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α-Amylases which catalyze the hydrolysis of starch with endo-acting property, are among the most important enzymes with widespread applications. α-Amylases can be found with different characteristics depending on the organisms they originate from. Tea plantation soil is intrinsically acidic and is a source of acidophilic or acid-tolerant bacteria. Bacillus sp. GUF8 was isolated from soil samples of a tea plantation from Guilan (Fooman) and identified as Bacillus cereus, based on 16S rDNA sequence and microbiological tests. This isolate neutralizes the acidic microenvironment by secreting urease and producing ammonia, as observed by pH indicators in nutrient plates and the urease test. After optimization of enzyme production, the α-amylase produced by the isolate was purified by concentrating the culture medium using acetone precipitation and then ion exchange chromatography (DEAE-Sepharose and UNOsphere Q columns) applied onto an FPLC system. This enzyme is active in a broad range of pHs (4.5-10) and temperatures (10-70˚C) with optimal pH and temperature at 6.0 and 50 ˚C, respectively. The effect of various metal ions and EDTA on enzyme activity was studied and its thermostability and kinetic parameters were also determined. Further experiments are currently under way to analyze the hydrolysis products of the amylolytic activity and determine the gene sequence of the enzyme.

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MOLECULAR WEIGHT DETERMINATION OF MULTIPLE MOLECULAR FORMS OF GLUCOSE 6-PHOSPHATE DEHYDROGENASE PURIFIED FROM STREPTOMYCES AUREOFACIENS

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Glucose 6-phosphate dehydrogenase (G6PD) of Streptomyces aureofaciens is a dual nucleotide specific enzyme utilizing NAD+ and NADP+ as coenzyme. Streptomyces aureofaciens G6PD was purified by ammonium sulfate (30-60%) and chromatography on DEAE-cellulose and Sephadex G100, up to 180-fold. Gel electrophoresis (SDS-PAGE & PAGE) was performed on purified enzyme having a specific activity of 3.2U/mg protein in order to determine the molecular weight of the enzyme subunits and the size of the isomers of the enzyme. Oligomeric forms of the active enzyme were determined by polyacrylamide gel electrophoresis in different gel concentrations (5.5-8.5 %) using activity staining method. Approximate molecular weights of the active bands were calculated from the relationship between retardation coefficients and the molecular weights of marker proteins ranging from 14 to 80 kDa using Ferguson plots .SDS- gel electrophoresis studies showed a single band corresponding to a M.W. of 51.5 kDa. Polyacrylamide gel electrophoresis of purified active enzyme indicated one major dimeric form of 105.0 kDa and 4 minor active bands belonging to higher oligomeric forms (trimer to hexamer) of 147.6 to 305.0 kDa .In conclusion,, the oligomeric forms of S. aureofaciens G6PD seems to be the result of subunit aggregations in the absence of SDS on polyacrylamide gel electrophoresis.

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PARTITION BEHAVIOR OF BACILLUS SUBTILIS ALKALINE PROTEASE IN AQUEOUS TWO-PHASE SYSTEMS

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Alkaline protease (EC 3.4.21.14) hydrolyzes proteins and breaks them down into more soluble polypeptides or free amino acids and enables the cell to absorb or adsorb and utilize the hydrolytic products. It is considered as a key player in many industrial applications and comprises two-thirds of the chemicals used in detergent industry. In this study, the partition behaviors of extracellular alkaline protease from Bacillus subtilis were investigated. An aqueous two-phase system (polyethylene glycol 10,000 (PEG10,000)/citrate) was examined with regards to the effects of sodium citrate concentration, PEG concentration and molecular weight of PEG on the partitioning of this enzyme. The best conditions for protease partitioning was 22 % (w/w) PEG and 18 % (w/w) citrate at pH 7.0, which showed a partition coefficient of 32.5, recovery of 162.5%, a protein yield of 97.01% and a purification factor of 21.12. The result indicated that the aqueous two-phase system (ATPS) is an effective technique as the first step in isolation and partitioning processes of alkaline protease.

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SERUM PARAOXONASE (PON1) AND ITS PHENOTYPE DISTRIBUTION IN CORONARY ARTERY DISEASE AND THE STUDY OF THE INHIBITORY EFFECTS OF SOME DRUGS ON SERUM ENZYME ACTIVITY

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Serum paraoxonase (PON1) is a HDL-associated enzyme which has different activities including paraoxonase and arylesterase activities. These activities are inversely related to the risk of cardiovascular disease. Because studies on the relationship between PON1 activity and the extent of coronary stenosis are very limited; in this study, 83 patients with angiographically documented CAD were investigated and were divided into two categories, with <50% stenosis and with >70% stenosis. Paraoxonase (with paraoxone as the substrate) and arylesterase (with phenyl acetate as the substrate) activities were investigated in the patients. The distribution of paraoxonase phenotypes and the inhibitory effect of 20 drugs on the enzyme activity were also determined. It was noticed that paraoxonase and arylesterase activities in patients with >70% stenosis (131.62± 71.8 and 77.62± 28.2, respectively) were significantly less than those with 50% stenosis (177.75± 83.6 and 93.91± 34.1, respectively). Upon calculation of the ratio of salt- stimulated paraoxonase to arylesterase activities, 34 patients were found to have AA phenotype (homozygous-low group), 36 had AB phenotype (heterozygous-high group) and 8 had BB phenotype (homozygous-high group). From 20 drugs studied, lorazepam had the most marked inhibitory effect on enzyme activity (52% inhibition in concentration of 10 μM). It was concluded that paraoxonase and arylesterase activities inversely associate with the extent of CAD. Reduced PON1 activity may play a role in the severity of coronary atherosclerosis.

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APPROACHING INULINASE TECHNOLOGY: ENZYME PRODUCTION BY ASPERGILLUS STRAINS

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Enzymatic hydrolysis of inulin is achieved in a single step by Endo- or Exo- inulinase (EC: 3.2.1.7 and 3.2.1.80, respectively). This is particularly significant in the production of High Fructose Syrups (HFSs) used in the food industry in various products such as ice creams, biscuits, cakes and soft drinks. In this study, five Aspergillus strains were chosen from the Persian Type Culture Collection (PTCC) according to literature, and the production of inulinase was tested through growth in a medium containing inulin as the sole carbon source. Chicory root extract was used as carbon source since it contains a high amount of inulin, as was confirmed by TLC. The five strains were cultured in chicory root extract medium and the mycelia were separated by filtration after five days. The production of enzyme was confirmed by SDS PAGE. Enzyme activity was assayed through measurement of the amount of reducing sugar (fructose) produced by the enzyme. This could be the initial step towards industrial production of inulinase for use in the food industry.

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THE EFFECT OF PH ON STRUCTURE AND FUNCTION OF CHOLINE OXIDASE

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Choline oxidase (E.C. 1.1.3.17) catalyzes the four-electron oxidation of choline to glycine betaine (N,N,N-trimethylglycine; betaine) via betaine aldehyde as an intermediate. The enzyme contains covalently bound FAD and utilizes molecular oxygen as primary electron acceptor. Among the members of the GMC oxidoreductase superfamily within which the enzyme can be grouped, choline oxidase is unique in that it catalyzes the oxidation of a primary alcohol substrate to a carboxylic acid via an aldehyde intermediate. The study of an enzyme involved in glycine betaine biosynthesis is of considerable interest for both biotechnological and biomedical applications, because recent studies have shown that accumulation of glycine betaine in the cytoplasm of cells allows growth in hyperosmotic environments of transgenic plants lacking efficient glycine betaine biosynthetic systems and of clinical isolates of a number of human pathogens. Despite a wealth of studies on the biomedical and biotechnological applications of the enzyme, minimal biophysical and biochemical studies on choline oxidase have been reported. Consequently, the analyses of pH dependence on structure and function of choline oxidase from Alcaligenes species based on the results of the experimental works, such as, Circular dichroism spectrum, DSC and spectrophotometric measurements are the main aim of this study and offer the opportunity to expand our knowledge about this enzyme. In this study we analyse the optimum pH of choline oxidase and its inactivation coefficient.

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COMPARISONS OF KINETIC PROPERTIES OF ACETYLCHOLINESTERASE FROM PHOSALON-SUSCEPTIBLE AND RESISTANT STRAINS OF COLORADO POTATO BEETLE

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Colorado potato beetle (CPB, Leptinotarsa decemlineata Say) is one of the most significant agricultural pests in the world,

responsible for extensive damage on potatoes. CPBs are resistant to all major groups of insecticides inculuding, cyclodienes, organochlorines, organophosphorous compounds (OPs), carbamates, and pyrethroids, and are notorious for quickly developing resistance to insecticides used for its control. A population collected from Dehpiaz (Hamedan, Iran) was found to be highly resistant to phosalon. An efficient mechamism of resistance to OP and carbamate insecticides is site-insensitivity at the target enzyme, acetylcholinesterase (AChE). Alterations in the structure of AChE can reduce the level of inhibition by these extensively used insecticides and confer resistance in insects and other arthropod species. Fourth instars (74-85 mg) were collected from each strain and starved for 24 h to remove gut contents. Each larva was cut in two pieces between the prothorax and mesothorax. The head and prothorax from individual larvae were homogenized in 0.01 M sodium phosphate buffer (pH 7.5) containing 0.1% (v/v) Triton X-100. The homogenate was centrifuged and the supernatant served as a crude enzyme preparation for the determination of AChE activity by the Ellman reaction using acetylthiocholine iodide (ATChI) as substrate. Protein concentration of samples was determined according to Bradford at 560nm, using bovine serum albumin as a standard. Mass homogenates of four Colorado potato beetle heads were prepared for Km and Vmax determinations in buffer/Triton X-100. The aliquots of each supernatant were incubated with .01 M DTNB for at least 15 min before assays. Values of Km and Vmax were determined at 25oC from AChE activities measured over 1 min for 15 different concentrations of ATChI ranging from 0.25 to 6.5 mM. Findings showed that Km and Vmax of susceptible and resistant strains were 5.48 mM; 277.77 µmol/L/min, 2.3 mM; and 175.44 µmol/L/min, respectively. Km in resistant strain was lower than that of susceptible strain. Nevertheless, Vmax was higher in susceptible strain. Activity of AChE in resistant strain was significantly higher than that of susceptible strain ($p<0.5$).

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ROLE OF N-TERMINAL RESIDUES OF L-ASPARGINASE II SIGNAL SEQUENCE OF ESCHERICHIA COLI IN GENERAL EXPRESSION AND EXTRACELLULAR TRANSPORT OF RECOMBINANT ENZYME

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L-asparginase II (amidohydrolase E.C.3.5.1.1) is used for the effective treatment of acute lymphoblastic leukemia (ALL). It catalyzes the conversion of L-Asn to L-asp and ammonia. By depleting serum asparagines, the enzyme reduces availability of this amino acid to malignant cells. Therefore, obtaining an expression system for extracellular recombinant enzyme will simplify downstream processing steps in biopharmaceutical production of this enzyme. Asparaginases II signal sequence contains 22 amino acids. The N-terminal segment of signal sequence contains hydrophobic and positively charged residues. Here, we sought to improve extracellular expression of this enzyme by substituting some of hydrophobic residues with amino acids presented in N-terminal segment of gram positive bacterial signal sequences. Upon sequence comparison, we substituted some hydrophobic residues with positively charged residues to increase the rate of protein transport to extracellular space. Genomic DNA was obtained from some laboratory strains of E. coli and ansB gene was amplified using gene specific primers. Amino acids were substituted during PCR with forward primer containing the desired substitution. Then, signal sequence mutated peptide was cloned into pET21 vector to compare overall expression and transport of signal-sequence variant with wild type ansB protein. Our results will lead us in developing new high efficient expression systems for this pharmaceutically valuable enzyme.

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THE EFFECTS OF VITAMIN E ON THE ACTIVITY OF SUPEROXIDE DISMUTASE ISOFORMS AND TOTAL ANTIOXIDANT STATUS IN RAT

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To protect against oxidative damage, organisms have developed a variety of antioxidant defenses. Beside endogenously specialized antioxidant enzymes, exogenous antioxidants such as vitamin E protect the organism against free radicals. To investigate the effect of vitamin E on the activity of the most important endogenous antioxidant enzyme, superoxide dismutase (SOD), we assessed the activity of its two isoforms , (SOD I, SOD II), and total antioxidant Status (TAS) in different time intervals and different doses of vitamin E to see if there is any synergistic effect in rat during six weeks. 28 male Sprague- Dawley rats were divided into four groups, the control and three dosing groups, receiving 10, 30 and 100 mg/kg of body weight daily of vitamin E. SOD I and SOD II activities were assessed in RBCs and TAS was measured in plasma at week 0, 2, 4 and 6. The activity of the two isoforms in the 30 and 100 mg/kg dosing groups increased significantly compared to the control group ($P <0.05$) but the TAS decreased significantly ($P < 0.05$) compared to the control group. In other words, despite increasing activities of the two isoforms of SODs, TAS decreased. This may be because of the pro-oxidant activity of vitamin E at higher doses because of a cumulative effect of the vitamin during the six week experiment.

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EFFECT OF DIFFERENT ORGANIC SOLVENTS ON KINETIC PARAMETERS OF FIREFLY LUCIFERASE

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Firefly luciferase is a hydrophobic enzyme and its activity depends on the type of solvent present in the reaction mixture. The influence of four organic solvents on the enzyme activity and stability were indirectly evaluated through the measurement of emitted light in the bioluminescence reaction, expressed in relative luminescence units (RLU). Solvents of used in the three groups were: ε increasing (formamide), ε decreasing (ethanol and ethylene glycol) and non-effective on ε (dimethyl sulfoxide). The reaction mixture used in the

bioluminescence measurements consisted of: Tris buffer (pH 7.75), adenosine triphosphate (ATP), Mg^{+2}, luciferin and a specific amount of the organic solvents. Optimum temperature, thermal stability, pH optimum, pH stability and residual activity of enzyme in different concentrations of mentioned organic solvents were measured. A comparative study on Km, Vmax and decay rate of enzyme in presence of organic solvents was also performed. While increasing concentration of both ε increasing and decreasing solvents relative to the buffer solution leads to a more decline in enzyme activity, addition of DMSO up to 25% (v/v) results in an increase in enzyme activity. There is also an increase in pH and thermal stability in the presence of DMSO.

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SEQUENCING AND CHARACTERIZATION OF AN EXTRACELLULAR α-AMYLASE FROM A BACILLUS SP. WHO

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Amylase (α-1,4-glucan-4-glucanohydrolase, EC 3.2.1.1), which catalyzes the hydrolysis of amylose, amylopectin, and related carbohydrates with endo-acting property on 1,4-glycosidic linkages, is among the most important industrial enzymes with widespread applications. A Bacillus sp.WHO, which produces an extracellular α-amylase, was isolated from the high natural radiation area in Ramsar.An α-amylase gene from this Bacillus strain was cloned in vector pTZ57R/T and its nucleotide sequence was determined. Also the whole sequence of this gene is outlined in GenBank with 929354 accession number. An open reading frame composed of 1596 bases, which encodes 530 amino acid residues, was found. This α-amylase gene shows high sequence homologies with other microbial amylase genes, such as Bacillus megaterium and Bacillus sp.WS06 (97% and 96% identity, respectively). The deduced amino acid sequence revealed four highly conserved regions of the α-amylase family. The native enzyme had a molecular mass about 58,000 daltons.The kinetic parameters and stability of this enzyme will be discussed.

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MITOCHONDRIAL NADH [RIGHTWARDS ARROW] NAD TRANSHYDROGENATION IN ADULT HYMENOLEPIS DIMINUTA

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Adult Hymenolepis diminuta mitochondria catalyze a transhydrogenation reaction between NADPH and NAD and between NADH and NAD. The NADPH [Rightwards Arrow] NAD reaction is catalyzed by an inner membrane-associated pyridine nucleotide transhydrogenase, whereas the NADH [Rightwards Arrow] NAD reaction is ostensibly catalyzed by another system(s). The source(s) of NADH [Rightwards Arrow] NAD activity was evaluated by assessments of its intramitochondrial distribution and thermal lability and by comparisons with the distribution/thermal lability of NADH dehydrogenase, lipoamide dehydrogenase, and NADPH [Rightwards Arrow] NAD transhydrogenase. The occurrence of NADH and lipoamide dehydrogenase components was readily demonstrable. Like NADPH [Rightwards Arrow] NAD transhydrogenase, NADH dehydrogenase was essentially membrane bound. Lipoamide dehydrogenase and NADH [Rightwards Arrow] NAD activities were, at different levels, in the membrane and soluble fractions. Based on thermal profiles, NADH and lipoamide dehydrogenase differed from each other and from NADPH [Rightwards Arrow] NAD transhydrogenase. Although the NADH [Rightwards Arrow] NAD profile closely paralleled that for lipoamide dehydrogenase, it also was similar to the NADH dehydrogenase profile. Collectively, these data are consistent with the supposition that the H. diminuta mitochondrial NADH [Rightwards Arrow] NAD transhydrogenation reaction is catalyzed by lipoamide dehydrogenase and possibly by NADH dehydrogenase rather than by an independent transhydrogenase system.

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EFFECT OF TYPE AND CONCENTRATION OF DIFFERENT SALTS AND STORAGE TIME IN REFRIGERATOR ON ANTIOXIDATIVE ENZYMES AND LIPID OXIDATION IN CAMEL, CATTLE AND CHICKEN MEAT

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Fat oxidation is a major factor in decreasing food quality especially in meat products. Living cells have several protective mechanisms against the oxidative process, including removal of peroxides by catalase and glutathione peroxidase (GSH-Px). Some commonly used food additives including different salts could compromise their antioxidative potential. Our objectives were to determinate the effects of NaCl and KCl at varying ionic strengths on catalase and GSH-Px activities and lipid oxidation in ground chicken, cattle and camel meat during refrigeration storage. Samples were selected from longissimus dorsi and breast muscles of 3 chicken, cattle and camel after rigor mortis. Meat samples were ground and divided into seven batches and each one was randomly assigned to one of the following treatments: no additive(control) , NaCl (0.625, 1.25 and 2.5%) and KCl (0.8, 1.6, and,3.2%). Samples were packed in plastic bags and stored at 4° C for 0, 2, or 4 days. Upon removal after each storage time, the samples were frozen at -70° C until analyzed. GSH-Px and catalase activities, TBA, fat content, peroxide, acid and iodine values were determined on all samples. Results showed that NaCl and KCl significantly increased TBA (lipid oxidation) but NaCl was more effective. GSH-Px activity decreased during 4-day storage in the presence of higher levels of salts. But catalase activity was stable during different conditions.There was a higher decrease in GSH-Px activity with NaCl than with KCl, whereas salt type had no consistent effect on catalase activity. TBA and GSH-Px had a significant correlation with salt type and ionic strength. Chicken samples had lower enzyme activities than cattle and camel. Ionic strength and storage time had positive effects on peroxide and acid values in 3 animal species. Samples had no

significant difference in iodine value. In conclusion, this study showed that rate of lipid oxidation and reduction in GSH-Px activity correlate with the ionic strength of the salt.

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THE ANTI-OXIDATIVE RESPONSE TO CADMIUM IN ARABIDOPSIS THALIANA

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In higher plants, heavy metals induce oxidative stress by generation of reactive oxygen species (ROS). ROS can rapidly attack all types of bio-molecules which will lead to metabolic dysfunction and cell death. Induction of antioxidant enzymes is an important protective mechanism that minimizes oxidative damage. We studied the effect of Cd stress on catalase, peroxidase, and polyphenol oxidase activities (as antioxidant), and also cell death in Arabidopsis thaliana in order to investigate its possible involvement in the generation of oxidative stress. Sterilized seeds were germinated on MS media supplemented with different concentration of Cd (0, 50, 85, and 100 µM).Three week-old seedlings were harvested for these studies. The activities of peroxidase and polyphenol oxidase had no significant difference among seedlings treated with 50, and 85 µM Cd and the untreated plants, but increased significantly at 100 µM Cd. Because of the participation of these two enzymes in H2O2 detoxification; apparently their activities increased due to additional H2O2 generation. Catalase activity in treated samples decreased as a result of its inactivation by cadmium. MDA level, an index of lipid peroxidation, did not show significant increase at 50 and 85 µM cadmium compared with control, but increased at the highest concentration of Cd (100µM). Staining with trypan blue showed that the leaves exposed to 100 µM Cd were more colored and had more dead cells. Increased leaf chlorosis was also correlated with increased Cd concentration. These results demonstrated that cadmium increases the activity of antioxidant enzymes and cell death in Arabidopsis thaliana. Therefore, Cd stress is a dangerous risk factor in plant nutrition.

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THE EFFECT OF COPPER ON ANTI-OXDATIVE ENZYMES AND CELL DEATH IN ARABIDOPSIS THALIANA

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Copper is an essential element for plant growth that is important in a wide range of biochemical and physiological processes. For example, Cu2+ is required as a cofactor of an isoform of superoxide dismutase (Cu/Zn). It also participates in electron-transfer reactions of photosynthesis (in plastocyanin). However, Cu2+ at high levels becomes strongly phytotoxic to cells and causes inhibition of plant growth or even death by induction of reactive oxygen species (ROS), and /or by interfering with the structure and function of proteins by binding to their sulfhydryl groups.. Plants minimize the diverse effects of ROS by anti-oxdative systems consisting of enzymatic and non enzymatic scavengers. We measured catalase, peroxidase and polyphenol oxidase antioxidant enzyme activities and cell death during plant encounter to excess copper. Sterilized seeds planted onto MS media completed with additional concentration of copper (0, 40 and 80 µM) and three week- old seedlings were used for our studies. Catalase activity significantly decreased in Arabidopsis thaliana treated with 40 and 80 µM of Cu2+ compared to control. This decline might be due to Cu2+ binding, replacing some other metal in the enzyme, or increasing H2O2 production that will inactivate catalase. Peroxidase activity was significantly increased at 80 µM Cu2+,t probably due to increased H2O2 production. Polyphenol oxidase activities were unchanged but Malondialdehyde content, index of lipid peroxidation, significantly increased at 40 and 80 µM excess copper compared to the control. Staining leaves with trypan blue showed the highest number of dead cells in excess copper (80 µM). Root growth was diminished strongly in copper treated plants (40 and 80 µM) due tof decreased cell division or cell elongation.

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SIMULTANEOUS ASSAY OF CELLULASE AND α-AMYLASE IN SOLUTION PHASE

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α-Amylases (EC 3.2.1.1) catalyze the hydrolysis of α- (1,4) glycosidic linkages of starch, amylose, amylopectin,glycogen, and various maltooligosaccharides.1,2 α-Amylases are produced from a wide variety of biological sources such as bacteria, fungi, plants, and animals. α-Amylases from different biological sources produce maltooligosaccharides of different sizes and amounts from starch and related materials due to a different number of D-glucopyranose binding subsites. Cellulases hydrolyze cellulose polymer to smaller oligosaccharides and glucose, and include three major types of enzymes including endoglucanases (EC 3.2.1.4) which randomly attack the cellulose polymer by endoaction, cellobiohydrolases (EC 3.2.1.91) which act as exoenzymes and remove cellobiose or glucose from the non-reducing end of the cellulose chain, and glucosidases (EC 3.2.1.21) which hydrolyze cellooligosaccharides and cellobiose into glucose. These enzymes can either be free, particularly in aerobic microorganisms, or grouped in a multi-component enzyme complex, cellulosome, such as in anaerobic cellulolytic bacteria. In this study we assayed the activity of cellulase alone and cellulase and α-amylase together.Also we measued the activity of α-amylase alone and α-amylase with cellulose. Furthermore the temperature and pH optima and the Vmax and Km of the enzymes were also measured. We observed that the optimum temperature and pH for the activity of cellulase alone and for cellulose + α- amylase were 60 ° C and 4.5, respectively. Values obtained for optimum temperature and pH of both α-amylase alone and α- amylase and cellulose together were 50° C and 6.5,respectively.

Metabolism

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GASTRIC SECRETIONS AFFECTED BY ESOPHAGEAL DISTENTION IN RATS

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Background: The effect of esophageal distention (ED) on gastric motility has been well documented, but very few investigations have been carried out on the effect of ED on gastric secretions. The aim of this study was to investigate the effect of esophageal distension (ED) on gastric acid and pepsin secretions and the mechanism(s) involved. Materials and Methods: Male adult Wistar rats (200-240 g) were anesthetized with urethane (1.2 g/kg, IP) and underwent tracheostomy and laparotomy. A catheter was inserted in the stomach through duodenum for gastric distention and gastric wash out.The esophagus was distended by a balloon (0.3 ml, 10 min). Gastric acid secretion was stimulated by either gastric distension (1.5 ml/100 g B.W.), pentagastrin (20 µg/kg, IP.) or insulin (0.6 IU/kg, IP.). Pepsin secretion was stimulated by carbachol (20 µg/kg, IP). Effects of cervical vagotomy and reserpine (1 mg/kg, IP.) were also investigated. Results: Distention, pentagastrin and insulin-stimulated gastric acid secretion were reduced by the esophageal distention ($P<0.001$, $P<0.05$ and $P<0.05$, respectively). Carbachol-induced pepsin secretion was also attenuated by the esophageal distention ($P<0.05$). Cervical vagotomy abolished the inhibitory effect of the ED on gastric distension-induced acid secretion. In reserpinized rats, ED reduced the basal gastric acid secretion ($P<0.05$). Conclusion: These results indicate that the vagus nerves are involved in the inhibitory effect of the esophageal distension on gastric secretory function.

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IN VITRO EFFECT OF QUERCETIN, MYRICETIN, KAEMPFEROL, RUTIN AND MORIN ON LDL GLYCATION

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The nonenzymatic glycation of LDL is a naturally occurring chemical modification of apolipoprotein B (apo-B) as a result of condensation between lysine residues and glucose. Glycated LDL is poorly recognized by LDL receptors and initiates different processes that can be considered proatherogenic. Thus LDL glycation may contribute to the increased atherosclerotic risk of patients with diabetes. The objective of this study was to investigate the effect of naturally occurring flavonols on LDL glycation in vitro. In this study LDL was isolated from EDTA-plasma by ultracentrifugation using a single step discontinuous gradient. Then glucose was added to LDL and LDL glycation level was estimated in absence and presence of quercetin, myricetin, kaempferol, rutin and morin by sodium periodate assay. This study showed that quercetin, myricetin, kaempferol, rutin and morin decreased LDL glycation in a dose dependent manner. Also it was demonstrated that these antioxidants decrease electrophoretic mobility of glycated LDL. The results show that quercetin, myricetin, kaempferol, rutin and morin y with their antioxidant properties probably inhibited LDL glycation and thus may have a role in ameliorating atherosclerotic risk of patients with diabetes mellitus.

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EFFECT OF EXTRA VIRGIN OLIVE OIL ON SERUM LIPOPROTEINS, ATHEROSCLEROSIS DEVELOPMENT AND LIPID PEROXIDATION IN CHOLESTEROL FED RABBITS

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In this study we report the effects of extra virgin olive oil on serum lipoproteins, lipid peroxidation, and progression of aortic lesions. A total of 20 male rabbits (five per each group) were fed for 12 weeks on a diet containing standard chow diet (S), cholesterol (C), extra virgin olive oil rich(O) and cholesterol + extra virgin olive oil (CO) . Fasting blood samples from heart were collected at the beginning, and the end of experimental period for serum lipoprotein and lipid peroxide determinations. The rabbits were killed after this period for histologic assessment of aorta. Means of serum lipids were not significantly different at the beginning of experimental period. After the experimental period, significant differences were observed in total cholesterol, HDL-C, triglyceride and MDA among groups. The comparison of C and CO groups showed that total cholesterol and triglyceride increases more in CO group (p was 0.02 and 0.007, respectively), however higher levels of MDA were observed in C group ($P< 0.003$) .Comparison of histologic results showed that atherogenic diets (C and CO groups) caused fatty streak development, however, cholesterol provoked a significant progression in lesion development, whereas diet enrichment with extra virgin olive oil prevented this progression ($p=0.02$). Such findings demonstrated the preventive effect of extra virgin olive oil against atherosclerosis development which is independent of plasma lipoprotein effect, and suggested that probably olive oil acts on arteries directly.

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RELATIONSHIP OF PLASMA GLUCOSE LEVELS TO SERUM LIPIDS AND APOLIPOPROTEINS IN MIDDLE AND OLD AGE FEMALES

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Objective: In this study, we evaluated the relation of plasma glucose level to lipid and apolipoprotein levels. Methods: Fasting blood glucose (FBG), high density lipoprotein cholesterol (HDL-C), apolipoproteins (apo) A1 and B100

levels were measured in 200 females aged 40-70 in Kerman society. The study population was divided into three groups based on the plasma glucose levels: 1. normal group (K1), 2.decreased glucose tolerance group (K2), 3.diabetes mellitus (DM) group (K3). RESULTS: Among 200 cases studied, groups K2 and K3 had more hyperlipidemia than group K1. The mean fasting serum triglyceride (TG) levels in groups K2 and K3 group were significantly higher than that of group K1 ($P < 0.01$), and the mean TG levels in groups K2 and K3 were higher than that in group K1 ($P < 0.05$). The serum apoB in groups K2 and K3 were significantly higher as compared with that in the normal group ($P < 0.05$). The correlation analysis indicated that there was a positive correlation of BG with serum TG in all groups and a negative correlation with HDL-C levels in groups K2 and K3 ($P < 0.05$). The plasma glucose levels were positively correlated with apoB100 level in groups K1, K2 and K3 ($P < 0.05$), and negatively correlated with apoA1 only in group K2 ($P < 0.01$). Conclusion: The results suggest that serum TG, TC, HDL-C and apoB100 levels are the main factors related to plasma glucose level.

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SEMINAL PLASMA LEVELS OF FREE 8-ISOPROSTANE, MALONDIALDEHYDE AND TOTAL HOMOCYSTEINE AND THEIR RELATIONSHIP WITH SPERM MOTILITY

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Poor sperm motility is the major underlying cause of male infertility of which the etiology is not completely elucidated. One of the factors that can potentially cause asthenozoospermia is oxidative stress. The aim of the study was to compare seminal plasma malonidialdehyde (MDA), 8-isoprostane, and total homocysteine (tHcy) levels in normozoospermic vs. asthenozoospermic males. A case-control study was designed. The case group consisted of 15 asthenozoospermic males. This group was compared with 15 normozoospermic men. Seminal plasma levels of 8-isoprostane and tHcy were measured using enzyme immunoassay (EIA) method. MDA level was determined by the thiobarbituric acid (TBA) assay. The data were expressed as mean ± SEM. Levels of MDA were higher in asthenozoospermic subjects than in control subjects (0.72±0.06 µM vs. 0.40±0.06 µM; $P<0.05$). No differences were seen in 8-isoprostane levels in asthenozoospermic subjects and controls (65.00±3.20 pg/ml vs. 58.17±4.12 pg/ml; $P>0.05$). Interestingly, tHcy levels were slightly higher in asthenozoospermic subjects than in controls (6.18±1.17 µM vs. 4.8±0.52 µM). Seminal plasma 8-isoprostane levels showed an inverse significant correlation with sperm motility ($r= -0.41$, $P<0.05$) and also with normal sperm morphology ($r= -0.42$, $P<0.05$). Seminal plasma levels of MDA showed an inverse correlation with sperm motility ($r= -0.53$, $P<0.05$). No relationship was found between MDA and normal sperm morphology. Seminal plasma levels of tHcy showed no correlation with lipid peroxidation. We concluded that homocysteine metabolism may not be impaired in asthenozoospermic males. However, a larger sample size is required to confirm these findings.

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SERUM LIPID OXIDATION KINETICS IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION AND HEALTHY CONTROLS.

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Introduction: Acute myocardial infarction (AMI) is associated with free radical formation and lipid peroxidation. The aim of this study was to examine, in-vitro copper induced lipid oxidation kinetics in unfractionated serum of patients with AMI and a group of healthy controls. Methods: sixty-four consecutive patients admitted to coronary care unit for suspected AMI, and 80 age-matched healthy control subjects were examined. Serum levels of lipids and lipoprotein lipids were determined. Lipid oxidation kinetics was estimated by monitoring the change of conjugated dienes in a 60-fold diluted serum after addition of 60 µM Cu2+. A number of quantitative parameters including lag-time, maximal rate of oxidation (V-max), and maximal amount of lipid peroxide products accumulation (OD-max) were evaluated. Results: The kinetic analysis showed a significantly shorter lag-time in patients with AMI (57 +/- 26 min, $p < 0.05$) than control subjects (65+/- 15), and significantly higher OD-max in control group (0.229 +/- 0.061 OD/unit) than patients (0.171 +/- 0.079, $p < 0.001$). A significant correlation ($p < 0.05$) was observed between the V-max and OD-max with total cholesterol and low density lipoprotein-cholesterol in patients and controls. Conclusion: Serum lipids in patients with AMI are more susceptible to initiation of in-vitro copper-induced oxidation than healthy controls. Rate and final extent of serum lipid oxidation in patients and healthy control, are associated with cholesterol and cholesterol-rich lipoprotein levels.

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EVALUATION OF SERUM LIPID PROFILES IN SUBJECTS WITH HELICOBACTER PYLORI INFECTION

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Helicobacter pylori (H. pylori) is the main cause of chronic gastritis, peptic ulcer, gastric cancer and lymphoma. Recent studies have indicated a relationship between chronic H.pylori infection and the prevalence of coronary heart disease (CHD).It seems that chronic H.pylori infection causes some changes in serum levels of lipids and lipoproteins.This study was designed to investigate the effect of chronic H.pylori infection on serum lipid levels. This cross-sectional study was

performed on 400 healthy volunteers refering to medical centers in Kashan, between 2005 and 2006. H.pylori IgG antibody was measured by ELISA and triglycerides, total cholesterol and HDL-cholesterol were measured by routine enzymatic methods. Prevalence of H.pylori infection was 79.8%.The serum triglyceride concentration and cholesterol/HDL-cholesterol atherogenic index were significantly higher in H.pylori seropositive individuals than in seronegative ones (p=.04 , p=.02 respectively). Chronic H.pylori infection may affect lipid metabolism in a way that could increase the risk of coronary heart disease.

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STUDY OF OXIDATIVE STRESS IN TYPE II DIABETIC PATIENTS AND ITS RELATIONSHIP WITH HBA1C

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Background: Hyperglycemia causes the long term complications in diabetic patients and may lead to death in many cases. Hyperglycemia increases the free radicals and reactive oxygen species that cause oxidative damage. Most of the oxidative effect appears in sensitive tissues such as kidney, heart and the eye. This study was aimed to evaluate the activity of antioxidant enzymes in diabetic patients and also to determine the correlation between hyperglycemia and lipid peroxidation. Methods: This study was carried out on 30 patients with type II diabetes and 30 healthy individuals as control group. Glycated heamoglobin (HbA1c) was measured as a marker of hyperglycemia using a chromatographic method (Biosystem) and malone dialdehyde was determined as a factor showing oxidative stress using a colorimetric method. Gluthatione peroxidase (GPX) and superoxide dismutase (SOD) activities were assessed using UV-Vis spectrophotometric techniques (Randox kit). Results: The mean of HbA1c was higher in diabetic patients compared to healthy group and the difference was statistically significant ($p< 0.001$). Serum MDA in diabetics was higher compared to those of healthy subjects ($p<0.001$). There were significant differences in activities of SOD and GPX between the two studied groups indicating lower activity in diabetic patients ($p<0.001$). There was a significant relationship between MDA and HbA1c in diabetic and healthy subjects. Conclusion: The obtained data showed increases in lipid peroxidation and oxidative stress in diabetes and also indicated a positive correlation between degree of hyperglyceamia and oxidative stress. Upon evaluation of oxidative status and selection of appropriate treatment measures, it would be possible to support antioxidant defense mechanisms in these patients Uperoxide.

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OXIDATIVE DAMAGE TO DNA AND LIPIDS:CORRELATION WITH PROTEIN GLYCATION IN PATIENTS WITH TYPE 1 DIABETES

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Diabetic hyperglycemia is associated with increased production of Reactive Oxygen Species (ROS). ROS reacts with DNA resulting in various products such as 8-hydroxydeoxyguanosine (8-OHdG) that excrte in urine due to DNA repair processes. Urinary 8-OHdG has been proposed as an indicator of oxidative damage to DNA. This study aimed to evaluate relationship between oxidative damage to DNA and protein glycation in patients with Type 1 diabetes. We measured urinary 8-OHdG level in diabetic patients and healthy subjects and discussed its relationship to glycated hemoglobin (HbA1c) and glycated serum protein (GSP) levels. Furthermore plasma malondialdehyde (MDA) level was monitored as an important indicator of lipid peroxidation in diabetes. We studied 32 patients with Type 1 diabetes mellitus and compared the measured factors with those of 48 age-matched non-diabetic controls. GSP and MDA were measured by colorimetric assay. Urinary 8-OHdG measurement was carried out using competitive in vitro enzyme-linked immunosorbent assay (ELISA). In the present study urinary 8-OHdG, HbA1c, plasma MDA and GSP levels were progressively higher in diabetics than in control subjects ($P<0.05$). Furthermore we found significant correlation between urinary 8-OHdG and HbA1c ($P<0.05$) in diabetic group.Correlation between fasting blood sugar and GSP or MDA were also significant. This case-control study in young diabetic patients showed that increased blood glucose and related metabolic disorders would result in oxidative stress and oxidative damage to DNA and lipids. Furthermore oxidative damage to DNA is associated to glycemic control level, while lipid peroxidation level was not significantly correlated with glycemic control level.

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EFFECT OF OPIUM ADDICTION ON LIPID PROFILE AND ATHEROSCLEROSIS ON NORMAL AND HYPERCHOLESTEROLEMIC RABBITS

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Atherosclerosis is the leading cause of mortality in the developed and some developing countries. Some people believe that opium consumption has beneficial effects for reducing blood sugar and lipids and can prevent heart disease. This will eventually lead to opium addiction in these people. In this study the effect of oral opium consumption on lipid profile and atherogenesis in normal and hypercholesterolemic rabbits was investigated. Thirty two male New Zealand White rabbits were used in this study. They were divided in four groups including; control, hypercholesterolemic, addicted, and hypercholesterolemic addicted, The animals were studied for three months. The blood samples were obtained and lipid profile was determined at the beginning of the study and at the

end of every month thereafter. After 90 days, the rabbits were sacrificed and the aortae were removed to check for lesion formation. The levels of cholesterol(C), triglycerides (TG), high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c), and atherogenic index (total C/HDL-c) in the hypercholesterolemic and hypercholesterolemic addicted rabbits were increased significantly (P<0.001). The increases in lipids and lesion areas in the aorta were higher in hypercholesterolemic addicted group than in the hypercholesterolemic group. Our findings suggest that oral opium consumption affects cholesterol metabolism and depending on the dietary condition can have an aggravating effect on atherosclerosis. The protective effect of morphine on cardiac disease, if any, is not probably due to the modulation of lipid metabolism.

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SERUM LIPID OXIDIZABILITY AND SEVERITY OF HEART MUSCLE INJURY IN PATIENTS WITH MYOCARDIAL INFARCTION

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Introduction: Myocardial infarction (MI) is associated with an acute phase response, and increased lipid peroxidation. Activities of serum cardiac enzymes in patients with MI are indicators for heart muscle damage severity. The aim of this study was to evaluate the relationship between serum lipid oxidation parameters and the levels of serum cardiac enzymes. Methods: sixty-four patients admitted to coronary care unit for MI were evaluated. Serum levels of lipids, lipoprotein lipids and cardiac enzymes were determined. Serum lipid oxidation profile was estimated by monitoring the production of conjugated dienes in 60-fold diluted serum after addition of 60 μM Cu2+. A number of quantitative parameters including lag-time, maximal rate of oxidation (V-max), and maximal amount of lipid peroxide products accumulation (OD-max) were evaluated. Results: The kinetic analysis showed that Lag-time was significantly correlated with cardiac isoenzyme of creatine kinase (CK-MB) (r=0.32, P=0.032). No significant correlation was observed between total CK and lactate dehydrogenase with lipid oxidation parameters. Conclusion: It seemed that the severity of heart muscle damage in patients with MI is associated with more resistance of serum lipids to in-vitro copper induced oxidation. This may be due to release of more acute phase reactant compounds with antioxidant activity in patients with more severe MI.

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PLASMA LIPIDS IN IRANIANS WITH SICKLE CELL DISEASE: HYPOCHOLESTEROLEMIA IN SICKLE CELL ANEMIA AND INCREASE OF HDL-CHOLESTEROL IN SICKLE CELL TRAIT

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The metabolism of lipids may be altered in sickle cell anemia and β-thalassemia patients. Plasma lipids in 24 patients with sickle cell anemia (SS), 15 patients with sickle/ β-thalassemia (ST), 19 individuals with sickle cell trait (AS) and 62 healthy individuals (AA) were measured. Total cholesterol concentrations in both sexes with sickle cell anemia and sickle/ β-thalassemia were lower (P <0.05) than AS and normal individuals. The mean HDL-cholesterol in males with SS and ST were (P=0.001) lower than AS males. However, the mean HDL-cholesterol in females with SS was lower (P <0.001) than AS females. The mean LDL-cholesterol of males with SS was lower (P <0.01) than AS and control males. Males with ST had a lower (P <0.001) LDL-cholesterol compared to control males. In females with SS the LDL-cholesterol was lower (P <0.001) than control females. However, females with ST had lower (P <0.05) LDL-cholesterol than AS and control females. There was no significant difference in total concentrations of cholesterol and triglycerides between males and females with AS and those with normal hemoglobin. However, the HDL-cholesterol in both genders with AS was higher (P <0.001) than normal subjects. Also, the concentration of LDL-cholesterol in both males and females with AS was lower than control males (P <0.05) and females. Hemolytic stress could be associated with a significant reduction in plasma lipids and lipoproteins. It appears that patients with sickle cell anemia and individuals with sickle cell trait are at a lower risk for coronary artery disease.

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THE ROLE OF THE STOMACH AND PROXIMAL INTESTINE IN THE REGULATION OF BLOOD GLUCOSE CONCENTRATION

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The effects of cholecystokinin peptides (CCK) as gastrointestinal hormones on intestinal glucose transport were investigated, largely using in vitro techniques. Measurement of transmucosal flux over 45 minutes with an established mini-Ussing chamber technique indicated that CCK33 and CCK4 had no inhibitory effect when added to the serosal compartment of paired mucosal sheets of guinea pig small intestine.CCK8 (the C-terminal octapeptide of CCK33) sulphated and non-sulphated and an analogue (FPL 1429 from Fisons) inhibited glucose and 3H-3-0-methyl-glucose transport from the luminal to the serosal chamber by up to 45% though the concentrations required to do so were high (i.e. greater than1 micro molar).The results indicate that there is a minimum amino acid sequence within the CCK octapeptide that is necessary for transport inhibition . A larger transport chamber technique was developed to measure glucose transmucosal and transcellular flux more accurately. Data from studies with CCK8 sulphate showed, by using each

mucosal sheet as its own control, that inhibition of transport is immediate within the limits of the experimental technique , and reversible. The analogue FPL 1429 KF appears to inhibit after a lag period, and CCK8 non-sulphate is inhibitory but only at a higher concentration than CCK8-sulphate. Despite containing a minimum sequence of inhibition of transport, the different peptides may bind to their receptors with varying affinities.

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STUDY OF SOME BIOMARKERS IN ACUTE MYOCARDIAL INFARCTION

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Several changes in serum biochemical factors occur in acute myocardial infarction (AMI). Recently alterations in serum levels of homocysteine (Hcy), sialic acid (SA) and hsCRP have been considered as risk factors and indices for prediction. This study was aimed to show the alterations in these factors and their relationships in AMI. Method: 34 patients with AMI confirmed by cardiologists were enrolled in this study. Also 51 apparently healthy individuals were selected as control group. Serum was prepared from all subjects in fasting state. Hcy and hsCRP were measured using ELISA and SA was determined by Ehrlich method. Results: In this study serum levels of Hcy, SA and hsCRP in AMI patients were 14.35±2.55µmol/l, 73.54±2.82 mg/dl, and 17.32±3.45 mg/dl, respectively while in the control group the respective values were 8.31±2.66 µmol/l, 59.82±2.70 mg/dl and 2.77±1.98 mg/l. Statistical analysis of data by t-test showed that serum level of Hcy, SA, and hs-CRP in the patients with AMI were significantly higher than those of control (P value <0.001). Also correlation was observed between Hcy and hs-CRP; and SA and Hcy. Conclusion: According to the obtained data we suggest a possible correlation between Hcy, SA, and hs-CRP and AMI. These factors can be used as biomarkers in AMI.

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IN VITRO PLASMA LIPID OXDIZABILITY IN OBESE AND NON-OBESE MEN

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Introduction: Obesity is associated with an increased risk of coronary heart disease, and it is characterized by dislipidemia. There is little available data about the oxidation capacity of plasma lipoproteins in obese subjects. So, we examined the susceptibility of plasma lipids to Cu-induced peroxidation in diluted plasma and the relationship between plasma lipids and body mass index (BMI) in a group of healthy men. Methods: In this case-control study the in vitro plasma lipid oxidizability in obese men (BMI≥25 kg/m2) was evaluated and compared with the control (BMI<25 kg/m2). Fasting plasma levels of lipids and lipoproteins were assayed by routine laboratory methods. Lipid oxidation was estimated by monitoring the change of conjugated dienes in diluted plasma after addition of Cu2+. The kinetic curves of the accumulation of lipid peroxide products were prepared, and a number of quantitative parameters including lag time, maximal oxidation rate (V-max), time of maximal oxidation rate (T-max), and maximal accumulation of absorbing products (OD-max) were evaluated. Results: TG and TC concentrations were positively correlated with BMI(r=0.4, p<0.01 and r=0.28, p<0.01, respectively).BMI correlates significantly with OD-max and V-max(r=0.23, p<0.05 and r=0.24, p<0.05 , respectively) Conclusion: Our results indicate that in vitro lipid oxidizability is significantly increased in obese subjects, and it is associated with triglyceride levels .Thus increase of lipid oxidation may be one of the mechanisms in obesity involved in the pathogenesis of atherosclerosis.

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UMBLICAL CORD LIPIDS AND LIPOPROTEIN (A) AND THEIR RELATIONSHIP WITH THE LIPIDS OF MOTHER'S BLOOD

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Introduction: Lipoprotein (a) [LP (a)] is a cholesterol-rich particle in human plasma and is considered as an independent risk factor for premature atherosclerosis and coronary heart diseases. Plasma levels of this lipoprotein are particularly affected by genetic factors. Some evidence showed that infants have lower serum concentration of LP (a) than adults. Materials and Methods: In this study plasma LP (a) was measured in cord blood from 58 newborns (32 girls and 26 boys) that were born in normal vaginal delivery. Also plasma lipids and lipoproteins of infants and their mothers were measured and their relationship was evaluated. Results: There was no significant association between blood cord LP (a) and total cholesterol (TC), triglycerides (TG), high density lipoproteins (HDL-C) and low density lipoproteins (LDL-C) in plasma of infants or their mothers. The mean of LP (a) in blood cord was 7.082±7.53 mg/dl and it was significantly higher in girls (8.96±8.28 mg/dl) than in boys (4.75±5.82 mg/dl)(p<0.05). There was a significant relationship between TC of infants and mothers' LDL and also infants' weight (r=0.29, p<0.05 and r=0.32, p<0.05, respectively). Conclusion: Results showed that plasma LP (a) in blood cord was not significantly correlated with infant and mother lipids. Distribution of LP (a) levels in newborns and adults is the same, but the mean of this lipoprotein in newborns was approximately 3 fold lower than that observed in adults.

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ENHANCEMENT OF WOUND HEALING BY TOPICAL APPLICATION OF NITRIC OXIDE (NO) COMPLEX

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It has been shown that nitric oxide (NO) plays a crucial role in wound healing. Either excessive or insufficient levels of NO may have serious consequences for certain tissues. Thus, exogenous or local delivery and manipulation of NO to the normal or impaired wound may be beneficial. The present study was designed to investigate the topical delivery of NO

via a NO-complex to the wound site. In this study 15 male Sprague Dawley (SD) rats were used and randomly assigned to either a control (n=6) or a treatment (n=9) group. Urine was collected at 24-hr intervals before wounding and throughout the course of the wound healing. On the day of wounding each rat received a 2×2 cm square full–thickness wound. On days 3, 7, 10, 14 and 21 wounds of treated rats received 200 mg of PEIC–NO complex and 200 µl of sterile PBS. Wounds of control rats received 200 mg of PEIC and 200 µl of PBS. The rate of wound healing was analyzed by VIA software. Blood pressure was measured before and after application of PEIC–NO. An unpaired two–tailed Student's t-test was used for data analysis and $p \leq 0.05$ was used to determine significance between groups. The mean (n = 9 days) pre–wound urinary NO3- output for NO–complex and control groups were 7.4±0.3 µmol/day and 6.7±0.34 µmol/day respectively. In the first three days following wounding urinary NO3- output in the control group nearly doubled from pre-wound levels to 12.4±2.4 µmol/day. However, PEIC–NO treated rats produced 22.5±1.1 µmol/day, a three fold increase over baseline levels and significantly more than controls during this period ($p \leq 0.09$). Based on percent of open wounds, the healing of wounds treated with topical PEIC–NO were significantly enhanced ($p \leq 0.05$) on days 7, 10, 17 relative to controls. The results of this study demonstrated that PEIC–NO can effectively provide controlled release of NO in vivo and will result in a significantly enhanced wound healing . Therefore, delivery of NO via NO-complexes in a controlled manner may have some promise in wound healing.

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AJUGA PLANT AND NEW ANTICOAGULATION

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Anticoagulants in blood used for transfusion during surgery and in hemodialysis may expose the patients to the risk of bleeding. However absence of effective anticoagulants may result in clotting of blood during surgery and hemodialysis and the treatment would be less effective as is the case where heparins, hirudin, prostacyclin and citrate are used as anticoagulants. Systemic anticoagulation can produce hemorrhagic complications in patients at high risk of bleeding. This study was aimed to isolate a new anticoagulant from Ajuga chemestus. This plant has been used as an anticoagulation and and antiplaque agent in Iranian traditional medicine. During our studies methanol extracts of the stalks, leaves, roots of Ajuoga chemestus has been studied for their biologically active compounds which can act either as an anticoagulant or enhance blood thrombosis.The methanol extract was added to fresh human blood and its anticoagulation and thrombosis activities were measured with light microscope.The results of this study suggest that Ajuga chemestus extract may be useful as an anticoagulant.

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DIFFERENT METABOLIC EFFECTS OF PHOSPHODIESTERASE INHIBITORS

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Phosphodiesterases (PDEs) are a grouop of enzymes with 11 families which selectively hydrolyze cAMP and cGMP. We have studied the metabolic effects of IBMX (a non-selective PDE inhibitor), amrinone, MC7 and MC9 (PDE3 inhibitors) in balb/c mice. The mice (10-12 g) were divided in groups of 5 to 10 with free access to water and food. Each mouse was weighed every day and left with no treatment (C1), or injected with normal saline (C2) or 1mg/kg drug for one week. Then the mice were sacrificed and their blood collected from the heart. The sera were separated and frozen at -18 ˚C for subsequent measuring of glucose, cholesterol and triglyceride concentrations. For measuring the glycogen storage, livers were separated and homogenized. There was no difference between growth pattern (GP) of male and female mice. Daily administration of normal saline decreased growth in the first 3 days. In the presence of amrinone, the first three days of GP was similar to the C2 and then increased. IBMX and MC9 did not change GP. MC7 in the first day extremely decreased GP and then returned it to the C2 levels. IBMX increased cholesterol, triglyceride, glucose and glycogen significantly, whereas amrinone decreased glucose, cholesterol and glycogen significantly. MC9 increased glycogen significantly. MC7 produced no significant effect. Stress decreases the growth. Adaptation to environment is produced after 3 days. In spite of similar inhibition effect, these agents have differential metabolic effects. Their mechanisms of action are not clear and require more investigation.

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ANTIAPOPTOTIC ROLE OF LUMINAL NADPH IN THE ENDOPLASMIC RETICULUM OF HUMAN NEUTROPHIL GRANULOCYTES

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Genetic deficiency of the endoplasmic reticulum glucose-6-phosphate transporter results in neutropenia and neutrophil granulocyte dysfunction in type 1b glycogen storage disease. Experimental inhibition of the transporter resulted in an increased apoptosis in differentiated HL-60 cells and human neutrophils; the effect could be prevented by NADPH oxidase inhibitor or antioxidants. It was supposed that microsomal glucose-6-phosphate transport has a role in the antioxidant protection of neutrophils, possibly through the substrate supply of the intraluminal NADPH generating hexose-6-phosphate dehydrogenase. To confirm this hypothesis in this study we have demonstrated the expression of hexose-6-phosphate dehydrogenase in human neutrophils. The presence and activity of the enzyme were shown in the microsomal fraction of the cells. The expression and activity of 11β-

hydroxysteroid dehydrogenase type 1, another NADP (H)-dependent microsomal enzyme responsible for cortisone-cortisol interconversion, were also detected in human neutrophils. The NADPH-generating cortisol dehydrogenase activity of the enzyme prevented neutrophil apoptosis provoked by the inhibition of the glucose-6-phosphate transporter. In conclusion, the maintenance of the luminal NADPH pool is an important antiapoptotic factor in neutrophil granulocytes.

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NADPH GENERATION IN THE LUMEN OF THE ENDOPLASMIC RETICULUM

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The lumen of the endoplasmic reticulum (ER) contains a separate pyridine nucleotide pool, which its redox balance is of vital importance. Reduced pyridine nucleotides – especially NADPH - are required for the functioning of intraluminal reductases, such as 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1). By now, hexose-6-phosphate dehydrogenase (H6PDH) has been regarded as the main intraluminal dehydrogenase responsible for NADPH generation. However H6PDH seems to be essential in the maintenance of intraluminal redox homeostasis, H6PDH knock-out mice are viable and devoid of serious pathological alterations. Therefore, it can be supposed that luminal enzymes other than H6PDH contribute to NADPH generation. We could show a significant isocitrate and a moderate malate dependent NADP+ reduction in rat liver microsomes. The isocitrate dehydrogenase activity was very likely attributable to the appearance of the cytosolic isocitrate dehydrogenase isozyme in the lumen. The isocitrate dehydrogenase activity of microsomes was present in the luminal fraction; it showed a strong preference towards NADP+ versus NAD+, and it was almost completely latent, i.e. the permeabilization of the membrane resulted in a more than tenfold elevation of the activity. Antibodies against the cytosolic isoform of isocitrate dehydrogenase immunorevealed a microsomal protein of identical molecular weight; the microsomal enzyme showed similar kinetic features and oxalomalate inhibition as the cytosolic one. In conclusion, we found that the lumen of the ER contains enzymes capable of NADPH generation, although the contribution of these enzymes to the in vivo NADPH generation needs to be further elucidated.

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EFFECTS OF LALLEMANETIA ROYLEANA L. SEED ON PLASMA LIPIDS CONCENTRATION IN RABBITS FED ON A HIGH CHOLESTEROL DIET

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Introduction: Hypercholesterolemia is one of the most important risk factors in ischemic heart disease. Several risk factors cause the development of atherosclerosis including: high levels of total cholesterol, LDL-C and triglyceride and low levels of HDL-C. Many species and herbs are known to have hypolipidemic effects. The present project studies the serum lipid lowering effect of Lallemanetia royleana L. seeds in hypercholesterolemic rabbits. Methods: The Lallemanetia rRoyleana seeds were purchased from Isfahan market and authenticated by botanists. The seeds were formed into powder and were mixed with rabbit standard foods. Rabbits were divided into 5 groups (n=5): normal control, hypercholesterolemic control (1% cholesterol) and three hypercholesterolemic treatment groups using diets containing 5%, 10% and 20% of the seed. The animals were fed for a period of 12 weeks. At the end of the treatment blood samples were obtained to analyze plasma cholesterol, triglyceride, low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C). Results: Plasma total cholesterol increased in hypercholesterolemic control group as compared with normal control group. Plasma total cholesterol and triglyceride decreased in all three hypercholesterolemic groups treated with Lallemanetia royleana seeds in comparison with hypercholesterolemic control group. The observed changes were not dose dependent. Changes in the distribution of cholesterol in HDL and LDL were found, so that the plasma LDL-C and HDL-C decreased significantly in all treated groups with respect to hypercholesterolemic control group. Conclusion: Our results showed that, although this plant decreased plasma cholesterol and triglyceride levels in hypercholesterolemic animals, it leads to an elevation of the atherogenic index.

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EFFECTS OF VANADATE AND OUABAIN ON NA+ DEPENDENT FATTY ACID ABSORPTION BY E.G.S

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Fatty acids are lipophilic molecules believed to pass easily across membranes, but studies have revealed that fatty acid transport occurs by special transporters present in many tissues. Further studies have shown that Na+ is also needed for this transport. In the absence of Na+, fatty acid uptake is significantly inhibited. To observe if this transport mechanism is affected by inhibitors of active transport, this study was designed and the rate of fatty acid uptake by E.G.S at different conditions was investigated. E.G.S (8-10 cm long) was prepared using rat jejunum filled with appropriate buffer. Sacs were incubated in a solution containing fatty acids (2mM) in which Na+ (100mM) was also present. The transport of fatty acids across the membrane was evaluated by measuring the level of fatty acids inside the sacs. In additional experiments the same procedures were performed except that vanadate (10mM) and/ or Ouabain (100uM) was also included in the solution. After 20 min. of incubation, fatty acids were measured in the buffer inside the E.G.S and compared with controls. Results showed that Na+ is essential for transport of fatty acids across the cell membrane, thus 300% increase was observed when Na+ was present .Ouabain and Vanadate

inhibited fatty acid uptake by 45 and 70 percent respectively. Many substances are absorbed across membranes by a co-transport mechanism in which Na+ is essential for the transport. Although most of this transportations are passive Na+- dependent processes, but inhibitors of active transport are also effective. The results of the present study showed that although Na+ presence is the essential part of this transport system , but vanadate and ouabain could also interfere with fatty acid transport presumably by inhibition of Na+ _ K+ ATPase in the membrane.

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ANTIHYPERGLYCEMIC AND ANTIHYPERLIPIDEMIC EFFECTS OFF MANGIFERA INDICAL.IN STREPTOZOCIN INDUCED ADULT MALE RATS

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Herbal medicine and medical plants such as Mangifera indica L. are widely used for the treatment of diseases like diabetes mellitus.we investigated the effects of the water extracts of Mangifera indica L. on serum glucose, triglycerides, cholesterol, LDL, HDL and the activities of aminotransferase enzymes in streptozocin-induced diabetic adult male rats. Continuous supplementation of this water extract by gavage at doses of 0.2, 0.5 and 1 g/kg in 0.5ml distilled water, resulted in a significant diminution of fasting blood glucose and triglyceride levels after 14 days. Levels of LDL and HDL and the activities of serum aminotransaminase enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), were changed not significantly in the extract supplemented group compared to control group.

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CHANGES IN METABOLISM OF LIPIDS FOLLOWING THE ADMINISTRATION OF ACUTE AND CHORNIC DOSES OF CHROMIUM

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Although chromium as a trace element in low concentrations is essential for the normal metabolism of lipids,but in higher concentrations causes disorders in lipids metabolism . To study the effects of chromium on some parameters related to lipid metabolism, different groups of rats were chosen and treated with chronic doses of 0.25, 0.5 and 1 mg / kg chromium for 75 days and with acute doses of 2.5, 5.0 and 10 mg / kg for 10 days. At the end of the experiments, rats were killed,blood samples were collected and analyzed for plasma lipid fractions. It was observed that in chronically treated animals the plasma levels of cholesterol, triglyceride and VLDL were decreased in a dose dependent manner whereas the plasma levels of fatty acids and lipoprotein lipase activity were increased. Therefore it seems that lipid metabolism in these animals was improved. Animals treated with acute doses of chromium showed an increase in plasma cholesterol and LDL and a decrease in triglycerides, VLDL and HDL concentrations. It seems that a decrease in HDL concentration was due to the inhibition of LCAT enzyme by high levels of chromium .In these animals the plasma levels of fatty acids and lipoprotein lipase activity were increased first but a decline in these parameters were observed with an increase in chromium dosage. Although chromium is essential for normal lipid metabolism, but low or toxic levels will cause disorder in lipid metabolism.

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REALATIONSHIP BETWWEN PLASMA GLUCOSE AND URIC ACID IN HYPERGLYCEMIC AND HEALTHY SUBJECTS

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It has been suggested that hyperglycemia increases purine metabolism. Enhancement of purine metabolism causes elevation in plasma uric acid that will result in the appearance of several cardiovascular risk factors such as arterial hypertension, hyperglycemia, and diabetes. The objective of this study was to evaluate the association between plasma glucose and uric acid. In a cross-sectional study, we measured glucose and uric acid in 160 subjects with hyperglycemia and compared them with 100 normal subjects in a population of Kerman, Iran. Results: The hyperglycemic subjects had increased levels of uric acid compared to the normal subjects ($p<0.05$). Conclusion: The present study shows a positive correlation between plasma glucose and uric acid.

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EVALUATION OF ANTIHYPERGLYCEMIC ACTIVITY OF SOME PLANTS DUE TO THEIR INHIBITORY EFFECTS ON ALPHA GLUCOSIDASE

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Diabetes mellitus is the most important disease with both genetic and environmental contributory factors, manifested by hyperglycemia. Diabetes affects nearly 10% of the population all over the world. Insulin therapy affords effective glycemic control, yet its shortcomings such as ineffectiveness upon oral administration, short shelf life,requirement of constant refrigeration, and, in the event of excess dosage, fatal hypoglycemia limits its usage. Treatment with other drugs such as sulfonylureas and biguanides is also associated with side effects. For various reasons in recent years, the popularity of complementary medicine has increased. Traditional plant therapies as prescribed by indigenous systems of medicine were used commonly. One hundred species of plants with known or unknown antidiabetic properties were collected and botanically identified. Some of these plants purchased from the Medicinal Herbal Markets in Kerman City. Methanolic and aqueous extracts were prepared by maceration method.α-glucosidase inhibitory effects were measured spectrophotometrically at pH 6.8 and 37°C using p-nitrophenyl- α-d-glucopyranoside as a substrate. One unit/ml

of the enzyme in 50mM sodium phosphate buffer and acarbose was used as a positive control. Among 200 extracts studied, 26 extracts showed more than 75% , 11 extracts 50-75% , 4 extracts 25-50% , 159 extracts 0-25% inhibitory effect on alpha glucosidase activity and none of the extracts showed any activating effects. In this study at least 21 plants were shown to have antihyperglycemic activities to be considered for further in vitro and in vivo studie.

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CORRELATION BETWEEN SEMINAL TOTAL ANTIOXIDANT CAPACITY AND SPERM LIPID PEROXIDATION IN THE FERTILE AND INFERTILE SMOKER AND NONSMOKER MEN

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Human semen contains a variety of enzymatic and non-enzymatic antioxidants t referred to as total antioxidant capacity (TAC) and has important role in free radical scavenging and normal sperm function. Malondialdehyde (MDA) is a stable product of lipid peroxidation (LPO) that is often measured as an index of oxidative stress. Objective: Detection of seminal TAC and MDA in four groups of fertile and infertile smoker and nonsmoker men and observation of correlations between these factors. Methods: Semen samples obtained from fertile nonsmoker (n=21), fertile smoker (n=25), infertile smoker (n=23) and infertile nonsmoker men (n=32) were analyzed according to WHO criteria. The levels of seminal TAC and MDA were measured by ferric reducing power of antioxidants (FRPA) and thiobarbituric acid reactive Substances (TBARS), respectively. Results: TAC level in seminal plasma of smoker men was approximately but not significantly lower than nonsmoker men, whereas this difference was significant between fertile and infertile men ($p<0.001$). MDA concentration in seminal plasma of smokers was approximately but not significantly higher than non smokers, whereas levels of MDA in seminal plasma of fertile men was significantly higher than infertile men ($p<0.001$). There was a negative and significant correlation between TAC and MDA levels ($p<0.01$) in seminal plasma. Conclusion: These findings suggested that human seminal TAC has a key role in free radical scavenging and it appears that its low content is related to high lipid peroxidation and abnormal sperm function. Thus its appears that a decrease in seminal TAC is a risk factor for idiopathic male infertility.

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THE EFFECTS OF COENZYME Q10 ON LDL OXIDATION IN VITRO

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The persent study investigated the effects of coenzyme Q10 on in vitro LDL oxidation quantitavely. It has been shown that coenzyme Q10 is able to inhibit CuSO4-induced LDL oxidation and increase the resistance of LDL against oxidation in vitro. The formation of conjugated dienes and thiobarbituric acid reactive substances (TBARS) were monitored as markers of the oxidation of LDL, respectively. Coenzyme Q10 also reduced electrophoretic mobility of the oxidized human LDL. Thus, we demonstrated that coenzyme Q10 exhibits strong antioxidant activity in CuSO4- mediated oxidation of LDL ($P<0.05$) in vitro. The inhibitory effects of the coenzyme Q10 on LDL oxidation was dose-dependent at concentrations ranging from 20 nM to 300nM; comparable to physiological concentrations. In conclusion, this study showed that coenzyme Q10 is a potent antioxidant in protecting LDL at physiological level.

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HE EFFECT OF PISTACHIO NUT POWDER ON PHOSPHATIDATE PHOSPHOHYDROLASE AND SERUM LIPID PROFILE IN RAT

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Phosphatidate phosphohydrolase (PAP) catalyses the dephosphorylation of phosphatidate to diacylglycerol and Pi. This reaction is a rate limiting and a regulatory step in glycerolipid metabolism .The produced diacylglycerol is a precursor to the synthesis of triglycerides and phospholipids. In this study the effect of Pistacia atlantica (belonging to Anacardiacea family) nut powder on PAP activity, liver triglyceride and serum lipid profile were investigated. Four groups of rats (n=6/group) were fed with normal diet or normal diet plus10% W/W of pistachio nut powder for 15 and 60 days. Then, PAP activity, triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol and liver triglyceride content were measured. Results showed that feeding rats with pistachio nut powder for 15 days had significantly elevated cholesterol in all lipoprotein fractions and reduced liver content of triglycerides ($P<0.05$). Although, PAP activity was reduced approximately 11%, it was not significantly different compared to the control group ($P>0.05$). On the other hand, rats fed for 60 days with pistachio nuts had no significant difference compared with the control group in all lipoprotein fractions ($P>0.05$), whereas the liver content of triglycerides significantly decreased ($P<0.05$). In addition, PAP activity also decreased nearly 16% with respect to the control group. The data show that in pistachio nut there is / are component(s) that increase cholesterol metabolism significantly in all lipoprotein fractions in the short term. Thus, in order to distinguish the effective substance of pistashio, it is necessary to obtain alcoholic and aqueous extracts of pistachio nut and to examine their effects on serum lipid metabolism separately,. On the other hand, in long term no significant difference was observed in cholesterol metabolism. This may be due to the effect of linoleic and linolenic acids in the pistachio nut that can reduce the elevated level of cholesterol in the long term. Considering that PAP is

involved in the formation of fatty liver and also the fact that pistachio nut has the ability in reducting the liver content of triglycerides by diminishing PAP activity in short and long periods, the pistachio nut can be used for the treatment of fatty liver through isolation and purification of its effective components.

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THE EFFECT OF POST HATCH FEEDING TIME AND STARTER PHASE LENGTH ON SOME BIOCHEMICAL INDICES OF BROILER CHICKEN'S BLOOD

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Broilers performance in the end of rearing may be influenced by post hatch feeding and the duration of feeding by the starter and/or grower diets. Those conditions also affect some biochemical indices of their blood, which in turn may finally affect their feeding and growth rate. This study was conducted with 720, Ross 308 broiler chicken to evaluate the influence of delayed access to feed and duration of feeding by starter and/or grower diets, in neonatal period. Four experiments were conducted to determine the effects of post hatch feeding time and starter diet phase length on the blood level of glucose, cholesterol, triglycerides and triiodothyronine in broiler chicken. Three fasting regiments were utilized in each experiment: 1) No fasting 2) 16 hours and 3) 32 hours fasting. Starter phases for each experimental group were 11, 16, and 21 days. Blood samples were taken at days 3, 21 and 42 after hatching. Sera were analyzed with auto analyzer for above mentioned parameters. Serum glucose value for the test birds was high in accordance to length of deprivation time. Levels of cholesterol and triglycerides were not affected meaningfully but serum triiodothyronine (T3) concentration, which may mediate some of the intestinal effects of feed deprivation were depressed in broilers without an access to feed.

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EFFECT OF TUMOR NECROSIS FACTOR ALPHA (TNF α) ON THE LEVEL OF PARATHYROID HORMONE

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Hyperparathyroidism is a disease in which parathyroid glands secrete more PTH than its normal amount because of an increase in the size of the glands, adenoma of the glands, or the synthesis and secretion of the hormone by tumors outside the glands. For example in some cases osteitis fibrosa cystica(Brown Tumor)could be a skeletal manifestation of an advanced hyperparathyroidism including parathyroid cancer. Therefore, it was anticipated that the immunologic system mediates a role between cancer and hyperparathyroidism. Previous studies have shown that exposure of the immunologic system with tumor cells results in an increase in the amounts of TNF alpha and interleukine1 (IL1). These factors stimulate hypothalamus to synthesize prostaglandins. There was also an increase in the activity of adenylate cyclase that converts ATP to cAMP. Secretion of PTH had a direct relation with the concentration of cAMP in parathyroid cells. Increase of PTH for a long period of time can cause osteoporosis, kidney stones, nephrocalcinosis, and infection of the urinary system. In the present study, TNF/alpha was injected intravenously and its concentration increased gradually in the serum of the rats. Then the concentration of PTH was measured in blood samples. Histochemical studies were also performed on sections of parathyroid gland. The results showed that an increase in the amount of TNF alpha can cause increased secretion of PTH and the latter causes an increase in the size and the numbers of parathyroid gland cells. The Golgi apparatus became larger and had more secretary vesicles than the control group. These observations demonstrated an increased function of parathyroid cells to secrete more hormone and have shown the effect of TNF/alpha on the increased secretion of PTH.

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DETECTION OF DIABETES MELLITUS AND HYPERLIPIDEMIA IN DOGS REFERRED TO MEHRSHAHR PET CLINIC

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In humans, diabetes mellitus is classified as type 1 or type 2 based on the pathophysiologic mechanisms and the pathogenic alterations affecting the beta cells. It is more clinically relevant and perhaps more accurate to classify diabetes in dogs and cats as IDDM or NIDDM rather than type 1 or type 2. Risk factors of diabetes mellitus include obesity, genetics, infection, immune mediated insulinitis, pancreatitis, drugs (glucocorticoids) and hyperlipidemia. Hyperlipidemia is a consequence of elevated plasma concentration of triglycerides and/ or cholesterol and is due to a disturbance in plasma lipoprotein metabolism. Lipoproteins are categorized into chylomicrons, VLDL, LDL and HDL. Primary forms of hyperlipidemia, are idiopathic and familial and the diseases associated with secondary hyperlipidemia include diabetes mellitus, pancreatitis, nephrotic syndrome, hypothyroidism and hyperadrenocorticism. Glucose and total lipids in blood serum of 100 dogs which referred to Mehrshahr Pet Clinic and also in some kennels in Mehshahr area were measured. Two cases with hyperglycemia and 11 cases with hyperlipidemia were found. There was a significant relation between hyperglycemia and hyperlipidemia and age group ($p<0.05$), but no significant relation was found between hyperglycemia and sex, breed and size of dogs ($p>0.05$). There were significant relations between hyperlipidemia and diet and size ($p<0.05$), but no significant relation was evidenced between hyperlipidemia and sex, age and breed ($p>0.05$).

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EFFECTS OF GLIBENCLAMIDE ON INSULIN SECRETION AND GLUCOKINASE ACTIVITY IN ISLETS OF LANGERHANS FROM NORMAL AND DIABETIC RATS

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Sulfonylureas such as glibenclamide (glyburide) have been widely used to treat type 2 diabetic patients for decades, but controversy remains about their precise mechanism of action. On the other hand, glucokinase serves as a glucose sensor in pancreatic β-cells and plays a key role in the regulation of insulin secretion and glucose homeostasis. The aim of the present study was to evaluate the effect of glibenclamide on insulin secretion and glucokinase activity in the rat isolated islets of Langerhans. The islets from normal and type 2 diabetic (nSTZ) rats were isolated by collagenase digestion method. Glucokinase activity was quantitated by measuring the rate of glucose-6-phosphate formation in the fluorometric assay. Insulin secretion from hand-picked islets was evaluated by static incubation technique. Insulin concentration was measured by rat insulin ELISA kit. Our findings obtained from incubation of glibenclamide with pancreatic islets showed that this substance increases basal insulin secretion (at 2.8 mM glucose) in both normal and diabetic rats compared with control (without drug) islets. However, the increase of insulin secretion in response to 16.7 mM glucose (GSIS) was not significant. On the other hand, glibenclamide had no activating and/or inhibiting effect on pancreatic glucokinase activity in both diabetic and normal Rats. But reduced activity of this enzyme in diabetic rats was significant in comparison to normals. These data show that increasing effect of Glibenclamide on insulin secretion is through a mechanism other than affecting GSIS. Morever, the regulation of pancreatic glucokinase does not depend on glibenclamide.

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RAMADAN FASTING EFFECTS ON THE SERUM PROTEINS AND LIPIDS

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Introduction: The aim of the study was to determine the variation of serum proteins and lipids in human subjects during Ramadan fasting. Materials and methods: The study group consisted of 50 male (35 ± 10 year old) volunteers. The lipids and proteins were determined on samples collected one week before Ramadan fasting, two and four weeks after the start of Ramadan and finally 2 weeks and 2months after the end of Ramadan. Measurement of serum proteins and lipids were performed using biochemical methods. Results: Our results showed statistically significant reduction of serum triglycerides and cholesterol during Ramadan fasting ($P< 0.05$). Protein variations were not significant. Conclusions: The data revealed that probably Ramadan fasting is a useful way for normalizing lipid profiles.

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METABOLIC SYNDROME, THE PROSPECTIVE DILEMMA OF IRANIAN HEALTH SYSTEM

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Objectives and background: The metabolic syndrome (MetS), posing the risks of cardiovascular disease and type 2 diabetes, is responsible for a growing number of premature deaths throughout the world. Although its exact pathogenesis is not clear yet, it is known as the second aim in cardiovascular risk factor control. Since the cardiovascular disease (CVD) is the primary clinical outcome of the metabolic syndrome, understanding its distribution in our society lead to better health planning for primary prevention of cardiovascular diseases.Materials and Methods: In a cross-sectional study implemented in rural and urban areas, 6400 people in Isfahan and Najaf-Abad and 6400 people in Arak were evaluated in phase I of Isfahan Healthy Heart Program (IHHP), Multi-stage lipids were measured under standardized conditions in the laboratory of Cardiovascular Research Center. Waist circumference was measured by tape measure and blood pressure by pressure gauge. Data was defined based on ATPIII's definition of metabolic syndrome. Systolic blood pressure > 140mmhg or diastolic pressure >= 90mmhg or consumption of drug were defined as hypertension. Data was analyzed based on SPSS9. Findings: OR Male n(%) Female n(%) Metabolic syndrome components (1.8-2.8)2.3 (2.2) 133 (4.9)312 BP/TG/HDL (1.8-3.9)2.7 (0.6) 38 (1.7) 108 BP/WC/HDL (0.9-1.6)1.2 (1.3) 80 (1.6) 101 BP/TG/FBS (4-6.7)5.2 (1.2) 71 (5.8) 368 BP/WC/HDL (1.1-2.7)1.7 (0.6) 34 (1.7) 108 BP/FBS/HDL (2.6-3.9)3.1 (2.5) 151 (7.5) 478 BP/TG/WC (3.6-7.7)5.2 (0.5) 33 (2.8) 178 FBS/HDL/WC (1.5-2.7)2.1 (1.3) 78 (2.6) 165 FBS/HDL/TG (2.5-4.1)3.2 (1.3) 33 (4.2) 268 FBS/WC/TG (6.4-8.6)7.4 (3.4) 210 (21) 1339 TG/HDL/WC (3.9-4.9)4.4 (8.1) 395 (28) 1795 Total Conclusion: These finding shows that Metabolic Syndrome prevalence in women is much higher than men in Iranian societies. Meanwhile the co-existence of decreased HDL and abdominal obesity in women are more than men. Considering the high association between Metabolic Syndrome and Cardiovascular disease and its mortality, the necessity of interventions for promoting lifestyle factors, such as adequate physical activities and healthy dietary programs, is obvious.

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GLUCOSE AND LIPID BLOOD LEVELS IN PATIENTS WITH FAMILY HISTORY OF CARDIOVASCULAR AND CEREBROVASCULAR DISEASES AND DIABETES MELLITUS

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Impaired glucose regulation and hyperlipidemia as components of metabolic syndrome (MS) are the results of

both: genetic predisposition and environmental influence. Family aggregation of these metabolic disorders is detected. Inheritance of components of MS is heterogeneous with complex genetic interactions. Aim and methods: We measured glucose, cholesterol and triglyceride blood levels in 103 patients with and without family history of hypertension (HT), diabetes mellitus (DM), cardiac deaths(CD) and myocardial infarction(MI) and stroke (S). We divided them in six groups: "A" patients with only HT in family history (FH)(15), "B" with only DM in FH(7), "C" with CD and MI in FH(22), "D" with S in FH(19), "E" with HT and DM in FH(11), "F" without observed diseases(29). The aim was to investigate the significant differences of biochemical parameters among these groups. Results: The means of glucose were normal in"A", close to critical level in"C"and"D" and increased($p<0.05$) in "B","E"and"F". There was no statistically significant difference in percent of patients with increased glucose, the highest percent was in "B" (57,1%) then in "F"(31%)and "E"(27.3%). The mean values of cholesterol were increased in "A" ($p<0.05$) "B"and"C" ($p>0.05$) and normal in others. Increased percent of patients with elevated cholesterol was detected in"A" (73.3%), "B" (71.4%) and "C" (59.1%)($p>0.05$). The means of triglycerides were increased in "A","B"and"D"($p>0.05$). The percent of patients with increased triglycerides was the highest in"A" (66.7%), "B" (57.1%) and"D" (31.6%)($p<0.05$). Conclusion: The importance of family history in discovering patients with the risk of metabolic disorders and the periodical control of the observed parameters in those patients is advised.

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S-ALLYL CYSTEINE SULFOXIDE (SACS) DECREASES INDUCED NITRIC OXIDE (NO) PRODUCTION AND CYTOTOXICITY IN A RAT INSULINOMA CELL LINE (RIN- 5F

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Inflammatory cytokines, especially Interleukin 1beta (IL-1 beta), are important mediators of beta-cell dysfunction. This has been attributed to increased production of nitric oxide (NO). S-allyl cysteine sulfoxide (SACS), also called allin, is an amino acid isolated from garlic. Since it has been shown that SACS has anti-diabetic effects, we studied the impact of SACS on IL-1 beta-induced NO production and cyto-toxicity in beta cell line (RIN-5F). Our results showed that increase in SACS concentration from 50 to 100 microgram/ml decreased IL-1 beta-induced NO production as measured by NO by product, nitrite, and increased the extent of [3-(4,5-dimethylthiazol-2-yl)]-2,5-diphenyltetrazolium (MTT) reduction activity assessed for cell viability in RIN-5F cells. A significant negative correlation was observed between IL-1 beta-induced NO production and cell viability in RIN-5F cells treated with SACS ($r=-0.79$; $P<0.01$). It suggests, at least in part, a NO mediated mechanism for SACS effects. These results are new insights, which may result in therapeutic value of garlic.

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PROTECTIVE EFFECTS OF NICOTINIC RECEPTORS ANTAGONISTS AGAINST ORGANOPHOSPHATES EXPOSURE IN PC12 CELLS

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Chronic and acute exposure to organophosphate (OP) pesticides may lead to persistent neurological and neurobehavioral effects, which cannot be explained by acetylcholinesterase (AChE) inhibition alone. It is suggested that other proteins are involved. Nicotinic acetylcholine receptors (nAChRs) are widely expressed throughout the peripheral and central nervous system. Although the effects of OP pesticides on muscarinic and muscle type nAChRs have been investigated extensively and are suggested to play a role in OP toxicity, the modulation of neuronal nAChR function by OP pesticides remains to be elucidated. The present study was performed to investigate the neuroprotective effects nAChRs antagonists against OPs exposure. The effects of nAChRs competitive antagonist mecamylamine and general antagonist dihydro β erythroidine (DHβE) were examined on expression and function of nicotinic receptors in PC12 cells exposed to different organophosphates. In this study, using northern blot analysis and reverse transcription-PCR we have shown that exposures of PC12 cells to organophosphates significantly decreased nAChRs both in mRNA and protein levels. In addition, the acetylcholinesterse activity and cells viability was significantly reduced time and dose dependently in cultured PC12 cells. Both mecamylamine and DHβE significantly reversed OPs inhibitory activity on nAChRs mRNA and protein level and cells viability. In contrast, reduced AChE activity was not compensated with these antagonists. This implicates that neuronal nAChRs are additional targets for some OP pesticides and nAChRs antagonists may play some protective roles in OPs poisoning.
Key Words: organophosphate pesticides, neuronal nicotinic acetylcholine receptor, PC12 cells, acetylcholinesterase.

p-281
EFFECT OF MORPHINE ON NT-3 GENE EXPRESSION IN THE NUCLEUS OF PARAGIGANTOCELLULARIS

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Morphine administration induces neurochemical adaptations in the noradrenergic system. The nature of signal responsible for opiate-induced adaptations of noradrenergic neurons in Locus coeruleus (LC) is not well defined. Neurotrophins-signaling pathways such as NT-3 (Neurotrophin-3) play a key role in regulating the noradrenergic response of LC neurons to opiates. The nucleus paragigantocellularis (PGi) is one of the major afferents to LC, so present study was designed to evaluate the expression of NT-3 in the context of acute and chronic administration of morphine in PGi nucleus. Rats in the acute morphine treatment group received 20 mg/ kg of morphine (i.p.) and in the chronic morphine treatment group, rats received 500 mg/ kg morphine (i.p.) for 3 days. PGi nucleus was collected 6 hours after this injection. PGi nucleus was assayed for the expression of NT-3 using semi – quantitative RT- PCR normalized to beta-actin gene expression. Results: Acute treatment of morphine did not change NT-3 gene expression. Chronic morphine administration altered NT-3 mRNA level in PGi ($p< 0.01$). In conclusion, the nucleus paragigantocellularis by neurotrophins signaling pathway may be involved in locus coeruleus activation.

p-282
RELATIONSHIP BETWEEN THE CLINICAL SCORING AND DEMYELINATION IN CENTRAL NERVOUS SYSTEM WITH TOTAL ANTIOXIDANT CAPACITY OF PLASMA DURING EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS DEVELOPMENT IN MICE

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Experimental autoimmune encephalomyelitis (EAE) is an animal model for multiple sclerosis (MS). EAE induced in a mouse strain (C57/BL6) to investigate the antioxidant status at various clinical stages of the disease in these animals. For this purpose, blood, brain and spinal cord samples were collected and examined at different scores following post-immunization with myelin oligodendrocyte glycoprotein (MOG). The clinical signs of mobility of animals on different days were associated with gradual increase in lipid peroxidation (LP) in brain and spinal cord. Changes in LP during EAE progression, was inversely related to superoxide dismutase (SOD) activity in erythrocytes. However, suppression of catalase activity in erythrocytes, tissue glutathione (GSH) and plasma total antioxidant capacity (FRAP assay) were the early events in EAE, occurred during scores 1 and 2. Biochemical alterations were corroborated with histopathological observations showing demyelination and inflammatory foci in central nervous system (CNS) of animals suffering from partial hind limb paralysis (score 3). These data suggest that generation of malondialdehyde (MDA) in CNS is a continuous process during EAE induction and suppression of antioxidant factors are early events of the disease, but crucial in increasing the vulnerability of CNS to demyelinating lesions.

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PROTECTIVE EFFECTS OF URIC ACID AGAINST EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS-INDUCED OXIDATIVE STRESS IN MICE

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Experimental autoimmune encephalomyelitis (EAE) is an animal model for multiple sclerosis (MS). EAE and MS are characterized by CNS inflammation, demyelination and neurodegeneration. Although the pathogenic mechanisms involved in EAE/MS are not well understood, accumulating data suggest that oxidative stress plays a major role in lesion development, since modulation of oxygen free radical production represents a new approach to the treatment of inflammatory and autoimmune diseases. Central nervous system tissue is particularly vulnerable to oxidative damage, suggesting that oxidation plays an important role in the pathogenesis of MS and EAE. To establish more conclusively whether uric acid (UA) is protective or therapeutic in EAE through inactivation of oxidant factors involved and sustain antioxidant capacity, we have assessed the effects of UA administration on several parameters relevant to the disease process and balance between oxidant and antioxidant system including Lipid peroxidation, superoxide dismutase and catalase enzyme activity, glutathione (GSH) and ferric reducing ability of plasma (FRAP) as well as Blood Brain barrier (BBB) permeability and also the clinical symptoms of EAE in the three interval (prior to, after onset and development) of disease. The results of the present study demonstrate an ameliorating effect of UA on oxidative damage in actively induced EAE in C57/bl6 mice and a significant increases in antioxidant factors in the EAE + UA group versus the EAE group especially at prior to or after onset of EAE (first and second group of this study).

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DIFFERENT CONCENTRATIONS OF FIBRILLAR Aβ 1-42 PROMOTES ADULT NEURONS INTO THE DIFFERENT PHASES OF CELL CYCLE

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The expression of cell cycle proteins in terminally differentiated neurons precedes cell death in Alzheimer's

disease (AD). This attempt by neurons to re-enter mitosis is a response to mitogenic stimuli like beta amyloid (Aβ). It was a question that in many areas of AD brains, cell cycle markers appeared without obvious plaque formation. On the other hand, cell cycling re-entry is not an immediate cause of cell death in AD. Instead the affected neurons live for many months before death with the Alzhimerous morphology such as cell cycle markers expression and tau pathology. Because abnormalities in mitotic mechanisms are early events in AD, we examined the possibility that picomolar levels of Aβ 1-42 could trigger the cell cycle re-entry. We also tested the effects of different concentrations of Aβ in promoting neurons to different phases of the cell cycle. Adult neuronal cultures were treated with Aβ 1-42. Cyclin D1 and B1 (G1 and G2 phase markers) were assessed by immunocytochemistry and apoptosis by TUNEL. Our data showed that reatment of neurons with toxic concentrations of Aβ resulted in extensive apoptosis whereas lower levels of Aβ promoted the neurons into G1 and G2 phases without noticeable apoptosis when compared with the toxic doses. In conclusion, present data, suggest that very low doses of Aβ induce neurons to re-enter the cell cycle. Different low concentrations of Aβ show variable progressing strength for promoting neurons to enter into different phases of the cell cycle and/or causing their death. Findings could explain why some neurons in AD degenerate while others just show elevated cell cycle markers and Alzheimerous pathology.

p-285
COMPARISON OF ABILITIES OF THREE DETERGENTS ON THE PURIFICATION OF MITOCHONDRIAL PORIN

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Solubilization of integral membrane proteins is a process in which the proteins and lipids that are held together in native membranes are suitably dissociated in a buffered detergent solution. The detergents for the purification of functionally active mitochondrial porin or voltage-dependent anion channel of the outer mitochondrial membrane are critically evaluated. In this study the effect of different families of detergents (Triton X-100, NP-40, and CHAPS) on the solubilization and purification of the pore-forming protein (porin) of the mitochondrial outer membrane of rat brain was investigated. For this purpose, after extraction of the rat brain we used porin purification using hydroxyapatite/celite column in three groups of CHAPS, NP-40, and Troton X-100. . After SDS gel electrophoresis and silver nitrate staining, western blotting was performed to confirm the identity of the protein we had employed. In group of CHAPS porin was not purified. In both Ttiton X-100 and NP-40 groups porin was purified, but silver staining and Western blotting techniques showed Triton X-100 was more efficient.In conclusion, Triton X-100 has more potential for porin purification.

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EFFECTS OF INTRA-AMYGDALA INJECTIONS OF NICOTINE AND GABA RECEPTORS RELATED AGENTS ON THE ANXIETY-LIKE BEHAVIOUR IN RATS

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Cholinergic and GABAergic systems are involved in neurobiology of anxiety. In the present study, we have investigated the effects and interactions of the nicotinic acetylcholine receptors (nAChRs) and GABAergic systems in the central amygdala of rats using the elevated plus-maze test of anxiety. Bilatral administration of nicotine (1 and 2 µg/rat) into central amygdala (CeA) produced a significant anxiogenic effect, shown by specific decreases in the percentage of open arm time (OAT%) and percentage of open arm entries (OAE%) and injection of Mecamylamine, a selective nAChRs antagonist (20, 30 and 50 ng/rat) produced significant anxiolytic behavior shown by increases in the OAT% and OAE%. The bilateral injection of the GABAA receptor agonist muscimol (0.25, 0.5 and 0.75 µg/rat) into CeA decreased OAT% and OAE% that are representative of anxiogenic–like behavior. Intra-CeA injection of receptors antagonist, bicuculline (0.25, 0.5 and 1 µg/rat) significantly increased anxiolytic behavior.Sub-effective doses of nicotine (0.25 µg/rat) when co administrated with muscimol did not increase the magnitude of anxiognesis significantly. Results suggest that GABAA and nicotinic receptors in CeA modulate anxiety behaviour.

p-287
CHANGES IN SERUM LEVELS OF MALONDIALDEHYDE IN PATIENTS WITH MULTIPLE SCLEROSIS: A RELATIONSHIP WITH TOTAL ANTIOXIDANT CAPACITY AND LIPID PROFILE

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Multiple sclerosis (MS) is an inflammatory, autoimmune, and demyelinating disease of the central nervous system (CNS). The etiology of MS has not yet been fully elucidated, but it is generally accepted that multiple factors are involved in the development of MS, such as genetic susceptibility, immunological mechanisms and environmental factors. Accumulating data indicate that oxidative stress (OS) plays a major role in the pathogenesis of MS. The present study was planned to investigate the relationship between plasma lipid

profile and antioxidant status in patients with MS. 74 patients with MS from the MS clinic in Isfahan were chosen. The control group was comprised of 74 healthy volunteers who were matched with patients for age and sex. Blood samples were used to evaluate total cholesterol and triglyceride levels as well as their lipoprotein fractions. Plasma total antioxidant capacity and malondialdehyde (MDA) were also determined. Our results showed that in the MS patients, total cholesterol, triglyceride, LDL-C and HDL-C did not differ significantly from control subjects.However the plasma VLDL-C in the MS patients was significantly different in comparison with control ($P<0.01$). Our results showed a significant increase in plasma MDA and also a significant decrease in plasma total antioxidant capacity in MS patients as compared with control. Conclusion: Our data indicated that there is a relationship between plasma antioxidant capacity and lipid profile in MS patients.

p-288
COMPARISON OF FIVE TRANSFECTION METHODS FOR GABAA RECEPTOR SUBUNIT GENES EXPRESSION IN HEK 293 CELLS

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By using five transfection methods, i.e., electroporation, modified calcium phosphate precipitation, Fugene (Roche), Escort (Sigma) and Effectene (Qiagen), transfection efficiency and expression intensity of GABAA reporter subunit genes were compared in HEK 293 cells in purpose to do path clamping. Of the five transfection methods employed, Fugene conferred the strongest expression of the GABAA receptor subunits genes with highest transfection efficiency and elctroportion provided the lowest expression. The results suggest that as far as transient gene expression is concerned for patch clamping, modified calcium phosphate precipitation would provide a useful and efficient means of GABAA receptor gene transfection to HEK 293cells.

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EFFECT OF INTRAVENTRICULAR MICROINJECTION OF W7 ON THE DEVELOPMENT OF TOLERANCE TO THE ANTINOCICEPTIVE EFFECTS OF MORPHINE IN ADRENALECTOMIZED RATS

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Objective: Calmodolin (CaM) play an important role in the development of tolerance to the antinociceptive effects of morphine. Adrenalectomy (ADX) potentiates the antinociceptive effect of morphine; however, the role of Hypothalamic–Pituitary–Adrenal (HPA) axis on this action has not been elucidated yet. So we examined the effect of W7 (specific Calmodolin inhibitor) on morphine induced analgesia in adrenalectomized (ADX) rats by tail-flick test. Material and Methods: male wistar rats weighing 250-300g were used. Tolerance to morphine was induced by daily injections of morphine (15 mg/kg/i.p) for 8 days. Adrenalectomy was performed after bilateral dorsal incision under general anesthesia with thiopental (15mg/kg/i.p). In sham operated animals only the incision was made but adrenals were not removed. Animals were allowed to recover from surgery for 5 days prior to initiation of experimental protocol. W7 (1 µmol/rat) was injected intracerebroventricularly (icv) concomitant with morphine (15 mg/kg) for 8 consecutive days. Tail flick latency (TFL) was used to assess the nociceptive threshold, at days 1, 3, 5 and 8 before and 30 min after morphine administration in sham operated and ADX rats. Results: daily morphine injection showed a marked analgesia in rats, but TFL decreased after 8 days, which shows the development of tolerance to morphine ($P< 0.005$). TFL following morphine treatment in ADX rats was significantly greater than sham operated rats ($P<0.005$) and W7 (1 µmol/rat/icv) significantly increased the antinociceptive effect of morphine in ADX rats compared to sham operated rats ($P<0.005$). Corticosterone replacement reversed the effect of W7 on ADX rats ($p<0.005$). Conclusion: the results of this study showed that HPA and Calmodolin may play a role in the development of tolerance to morphine antinociceptive effects in rats. Further studies are needed to elucidate the underlying mechanisms.

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DETERMINATION OF INTRAVENTRICULAR MICROINJECTIONAL EFFECTIVE DOSE OF W7 IN ADRENALECTOMIZED ADULT MALE RATS: (EFFECT OF INTRAVENTRICULAR MICROINJECTION OF W7 ON THE DEVELOPMENT OF TOLERANCE TO THE ANTINOCICEPTIVE EFFECTS OF MORPHINE)

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Objective: Calmodolin (CaM) play an important role in the development of tolerance to the antinociceptive effects of morphine. Adrenalectomies (ADX) potentiate the antinociceptive effect of morphine; however, the role of Hypothalamic–Pituitary–Adrenal (HPA) axis on this action has not been elucidated yet. So we examined the effect of W7 (specific Calmodolin inhibitor) on morphine induced analgesia in adrenalectomized (ADX) rats by tail-flick test. Material and Methods: male wistar rats weighing 250-300g were used. Tolerance to morphine was induced by daily injections of morphine (15 mg/kg/i.p) for 8 days. Adrenalectomy was performed after bilateral dorsal incision under general anesthesia with thiopental (15mg/kg/i.p). In sham operated animals only the incision was made but adrenals were not removed. Animals were allowed to recover from surgery for 5 days prior to initiation of experimental protocol. W7 0.25, 0.5, 1 and 2 µmol/rat was injected intracerebroventricularly (icv) concomitant with morphine (15 mg/kg) for 8 consecutive days. Tail flick latency (TFL) was used to assess the nociceptive threshold, at days 1, 3, 5 and 8 before and 30 min after morphine administration in sham operated and ADX rats. Results: daily morphine injection showed a marked analgesia in rats, but TFL decreased after 8 days, which shows the development of tolerance to morphine ($P< 0.005$). TFL following morphine treatment in ADX rats was significantly

greater than sham operated rats (P<0.005) and W7 (1 and 2 µmol/rat/icv) significantly increased the antinociceptive effect of morphine in ADX rats compared to W7(0.25 and 0.5 µmol/rat/icv) in ADX rats and sham operated rats(P<0.005) . Conclusion: the results of this study showed that HPA and Calmodolin may play a role in the development of tolerance to morphine antinociceptive effects in rats.the data indicate that intraventricular microinjectional effective dose of W7 in adrenalectomized adult male rats is 1 µmol/rat. Further studies are needed to elucidate the underlying mechanisms.

p-291
THE ROLE OF RO15-4513 DRUG IN BLOCKING THE EFFECTS OF ETHANOL ON FIELD-MOUSES

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Background: RO15-4513 is a laboratory drug and a weak inverse agonist of the benzodiazepines.the behavioral effects of ethanol is similar to tranquillizing effects of benzodiazepines. Objective: we have an intention to prove the role of RO15-4513 in prevent the effects of ethanol on consciousness. Design: from 40 ethanol intoxicated field-mouses :30 were injected with RO15-4513 and 10 were injected with a neuter solution(to create same conditions in experimental environment such as injection vasovagal effects and so on...).Results: 30 mouses that injected with RO15-4513 beginning to become conscious and after 2-4 minutes all of them were sober.other 10 mouses yet were unconscious. Conclusion: RO15-4513 could block and inhibit the effects of ethanol on consciousness. Drug mechanism (as a brief): this laboratory drug(RO15-4513)blocks the effects of alcohol on GABAa complex receptors.

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CHRONIC EFFECT OF GABAPENTIN ON LIVER FUNCTION

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Objective: Gabapentin (GPN) is a new antiepileptic agent currently in used as add-on therapy in adult patients suffering from partial seizures resistant to conventional therapies. Material AND Method: Adult male (Wistar) rats were administered intraperitoneally with Gabapentin [20mg/Kg body weight or 100mg/Kg body weight daily] for 45 days. After the experimental period, the liver function tests were carried out in control and experimental groups using autoanalyser (RA1000 Japan). Results: The activity of liver enzymes, with 20 mg/kg B.Wt. of GPN were not significantly different from the control group but, the serum levels of various enzymes such as AST, ALT, ALP, LDH, direct Bilirubin and total Bilirubin were enhanced significantly with 100 mg/kg B.Wt. of GPN. However, total protein and Albumin decreased in this group as compared with control animals. The histopathology of the liver parenchymal cells also showed minute foci of necrosis in few rats treated with high dose of GPN; Whereas, in therapeutic dose it showed almost normal. Conclusion: At therapeutic dose Gabapentin is a safe drug with regards to liver function & hepatocellular damage. Although liver enzymes significantly elevated in high doses of GPN, and has a minimal necroinflamatory effect on liver tissue, GPN can be a drug of choice for epileptic patients.

p-293
LOCALIZATION OF EPIDERMAL-TYPE FATTY ACID BINDING PROTEIN IN MACROPHAGES IN SCIATIC NERVE AFTER CRUSH INJURY

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The regeneration of axon and myelin sheet after crush injury of peripheral nerves involves interaction of several types of cells, including Schwann cells, monocyte, macrophage and fibroblast. Among them, haematogenous macrophages invading into the peripheral nervous system play a major role in myelin uptake during Wallerian degeneration. The localization of epidermal-type fatty acid binding protein (E-FABP) in the mature mouse sciatic nerve after crush injury was examined by immuno-light and electron microscopy. Numerous macrophages immunopositive for both anti-E-FABP and F4/80 a macrophage marker, were found in degeneration process of sciatic nerve. Macrophages contained phagosomes of various sizes and they were regarded as those actively involved in the phagocytosis of sciatic nerve debrise. The present detection of E-FABP immunopositivity selectively in invading macrophages suggests possible involvement of E-FABP and/or its ligand fatty acids in the process of peripheral nerve regeneration.

O-294
EVALUATION OF APOPTOSIS IN HIPPOCAMPAL REGION OF DIABETIC TYPE I RATS BY REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION (RT-PCR)

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Diabetes is a metabolic disease which is often accompanied by cognitive deficits and modifications of hippocampal function and plasticity. We examined the occurrence of apoptosis in hippocampus of diabetic rats and also the effect of diabetes on expression of Bax and Bcl-xS genes which are promoters of apoptosis, as well as of Bcl-2 and Bcl-xL which are inhibitors of apoptosis. We selected twenty four Wistar rats; half of them were made diabetic by intra-peritoneal administration of a single 60 mg/kg dose of streptozotocin (STZ) while the others received normal saline and served as control. Using RT-PCR, we measured the changes in Bcl-2, Bcl-xL, Bcl-xS and Bax mRNA levels in hippocampus of both groups. After eight weeks, the diabetic group had significantly lower body weight and higher blood glucose levels in comparison with the control group (P<0.01). mRNA levels of Bcl-2 and Bcl-xL were lower

in hippocampus of diabetic group than of the control group, whereas expression of Bax and Bcl-xS mRNA in hippocampus of diabetic rats were higher than the controls ($P<0.01$). Therefore, the induction of diabetes is associated with increased ratios of Bax/Bcl-2, Bcl-xS/Bcl-xL and Bax/Bcl-xL in hippocampus which shows that apoptosis is favored in hippocampal region.

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ASSESSMENT OF NEUROTROPHIC FACTORS GENES EXPRESSION IN BONE MARROW STROMAL CELLS

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Bone marrow stromal cells (BMSCs) are multipotent and their unique characteristics make them promising candidates for clinical applications. BMSCs contain mesenchymal stem cells and progenitor cells can be expanded rapidly in vitro and have the potential to be differentiated into neuronal and glial cell types under experimental conditions. Evidence accumulated over the past decade suggests that the neurotrophic factors play an important role in differentiation of these BMSCs into neurons and promote neuronal survival and stimulate axonal growth. The objective of this study was to determine whether the rat BMSCs express some important mammalian neurotrophin family such as Bain Derived Neurotrophic Factor (BDNF), Nerve Growth Factor (NGF), Neurotrophin 4 or 5(NT-4/5) and Glial Derived Neurotrophic Factor (GDNF). For this purpose, BMSCs were harvested from femur and tibia of adult rats and cultured in αMEM supplemented with 10% fetal bovine serum. The passage 5 of the BMSCs was assessed for expression of BDNF, NGF, GDNF and NT4 using RT-PCR and immunocytochemistry. The molecular study on these BMSCs showed that BDNF, NGF, GDNF and NT4/5 genes have been expressed definitely.

Plant Biochemistry

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THE STUDY OF SHOOT AND ROOT INDUCTION RESISTANCE TO KANAMYCIN IN THREE SPECIES OF ARTEMISIA

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Plants of genus Artemisia produce many essential oils from the group of sesquiterpene lactones that exhibit the highest antibacterial activity and anti-inflammatory properties. Three species of Artemisia (A. annua, A. sieberi and A.aucheri) were selected in this study. Artemisinin is a new effective antimalarial drug extracted from A. annua. Santonin is an antimicrobial drug extracted from A. sieberi and A. aucheri. In recent years there is more progress in the molecular regulation of artemisinin biosynthesis and other secondary metabolites, which were named metabolite engineering. In this way, genes of the key enzymes involved in biosynthesis of secondary metabolite should be cloned and transferred in to plant. By genetic engineering we can over express the key enzymes in biosynthesis pathway. Often for the selection of transformed plants, selectable marker such as NPT II gene was used as a selectable marker for kanamycin resistance plant cells. In this study 5 concentrations of kanamycin (2.5, 5, 10, 15 and 20 mg/l) were used as well as one 0 concentration of kanamycin as control in shoot and root induction mediums of every three Artemisia species. Shoot and root induction of every three Artemisia species were completely inhibited at 5 mg/l kanamycin but in control samples regenerated plants were obtained.

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TISSUE CULTURE, REGENERATION AND TRANSFORMATION STUDY OF ARTEMISIA SIEBERI VIA AGROBACTERIUM TUMEFACIENS

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Artemisia sieberi is an aromatic perennial plant, which produce many secondary metabolites such as santonin, camphor and cineol. This plant was applied as dominant plant in semi desert regions in Iran and many other part of the Asia. Because of their tolerance to wind erosion, they are very important in preservation of soil. Newly Pharmaceutical aspects like anticancer and antibacterial activities were reported for this plant. There is no report in tissue culture, regeneration and transformation of it. For the regeneration of A.sieberi, one month aseptic plants leaves were used as explant in various concentrations of hormones including NAA, BAP, IAA, 2,4-D and picluram.. The statistical analysis showed that the treatments containing 2 mg/l BAP, 0.05 mg/l NAA and 2 mg/l BAP, and 0.5 mg/l IAA were best regeneration medium. For root induction, 0.05 mg/l NAA was suitable. For transformation of leaf explants from one month plant, we used Agrobacterium tumefaciens strains LBA4404 and GV3101 including pBI121 plasmid harboring gus gene with CaMV35S promoter and nos terminator. Also we used A. tumefaciens strain LBA4404 including pZGA22 plasmid harboring gus gene with CaMV35S promoter and g7 terminator. Regeneration medium containing 2 mg/l BAP and 0.05 mg/l NAA was used for regeneration of transgenic plant that regenerated in selective medium including antibiotics. Presence of the transformed gene was approved by PCR with specific primers for gus gene and gus assay. Highest rate of transformation with A. tumefaciens strain LBA4404 plasmid was obtained.

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THE BENEFICIAL EFFECTS OF UNRIPED MOMORDICA CHARANTIA FRUIT ON SOME BLOOD BIOCHEMICAL PARAMETERS IN RATS

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Introduction: Momordica charantia (MC) belongs to the cucurbitaceae family. MC is widely used in traditional medicine as an antidiabetic agent. The aim of the present study was to evaluate the effect of the unriped fruit of MC on some blood biochemical parameters in rats.Materials and Methods: Three groups of rats received a diet without (control) or with 1% or 2% MC for two months. Blood samples were collected at the beginning and the end of the study.Sera were separated and used for the determination of biochemical parameters including fasting blood sugar (FBS), glycated hemoglobin (HbA1c), cholesterol (Cho), high density lipopreteins (HDL), low density lipoproteins (LDL), triglycerides (TG), urea (U) and creatinine (C).Results: The diet containing 1% and 2% MC decreased FBS and HbA1c significantly.Compared to the control group.The 2% MC diet decreased Cho and LDL compared to control group. There was no significant change in other factors. Discusion and conclusion: The findings suggest that MC can effectively normalize glucose and lipids in patients.Clinical trials are recommended.

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A COMPARISON STUDY ON ANTITUSSIVE EFFECT OF CORDIAL MYXA AND ZIZIPHUS SPINA-CHRISTII EXTRACT IN RATS

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Traditionally the fruit of Cordial myxa is used for lung disorders including coughing. The use of fruit of Ziziphus spina-christii is common in Khoozestan area. The study aimed at evaluating anti-inflammatory effect of fruit extract of Cordial myxa and examining the antitussive effects of Ziziphus spina-christii. After preparation of hydroalcoholic extract of fruits, the rats were divided into 4 groups (n=6). Grooup 1 received normal saline as control intra-peritoneal (IP). Group 2 received codeine phosphate (IP), group 3 received the extract of Cordial myxa and group 4 received the extract of Ziziphus spina-christii. Then the rats in each group were exposed to CO2 in closed apparatus for ten min. The mean of cough in each group was calculated and compared to other groups. The mean of cough in group 1 to 4 respectively was 139, 26.2, 60 and 86.2. The mean of cough was significantly different between group 1 and other groups. It was not significantly different between groups 2 and 3, but was different between 2 and 4. Thus, the extract of the fruit of Cordial myxa had better antitussive effect than Ziziphus spina-christii.

p-300
STS-PCR, USEFUL METHOD FOR DETECTION OF HMW SUBUNITS IN WHEAT

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Since quality in hexaploid wheat (Triticum aestium L. em Thell.) is a very complex trait, which is responsible for the elasticity and chohesiveness (strength) of dough, it is an important determinant of bread making quality. Good or poor wheat bread making quality is associated with two allelic pairs at the Glu-D1 complex locus designated 1Dx5-1Dy10 and 1Dx2-1Dy12, respectively. Probing these subunits have been conducted by SDS-PAGE method before. In this study we have reported the development of an alternative screening method based on glutenin genes themselves using the polymerase chain reaction (PCR). After adjusting the approach on some well-known Iranian hexaploid cultivars, we analyzed some of the lines of gene bank. In the next step, DNA samples of the lines were extracted, and using specified primers for 1Dx5-1Dy10 and 1Dx2-1Dy12 pair of alleles the PCR analysis was done. The efficiency of paired, specific primers for 1Dx5 allele was validated using some commercial wheat for which the existence of 1Dx5 allele was confirmed. After the validation of the efficiency of paired primers by commercial wheat cultivars, a SDS-PAGE was performed on bread wheat native lines proteins. In the next step using above primers and PCR analysis on wheat native lines of genetic resource unit of the Directorate of Wheat Research Karaj, the given alleles were probed. The utilization of paired specific primers for 1Dy10 and 1Dy12 also resulted in the multiplication of 567 paired base segments specified for 1Dy10 allele, indicating the correlation between these two alleles. PCR analysis showed no bared with 1Dx6 primer. Protein subunits in migration SDS-PAGE is not always correlated with their molecular weights, making the selection of parental lines a difficult task in breeding programs. The results of this study showed that the misleading results of a glutenin heavy subunits SDS-PAGE analysis could be avoided by PCR. The precision of this method was confirmed for all cultivars both in the absence or presence of 1Dx5, 1Dy10 and 1Dy12. The dissimilarity of SDS-PAGE methods with that of PCR could be resulted from the existence of within line biotypes where in conventional methods of wheat breeding is unavoidable. Biotypes even were present among some common cultivars. The relatedness of alleles may further be ideal by usage of primers. The increased information about sequences and PCR results could lead into more precisely identification of novel alleles. 1Dx5*, 1Dx2.2*, 1Dx2.2, 1Dx4, 1Dx3, for example are not the transformations of 1Dx5 as 1Dx5 specific primers did not multiply the 450 paired based segment of interest. More alleles are needed to understand whether these novel alleles are closer to 1Dx2 gene or not. Precision, rapidity, simplicity, as well as avoiding toxic chemicals such as acryl amide made PCR a reliable substitution for standardized methods of selecting genotypes with 1Dx5 and other genes. With PCR, furthermore, it is possible to screen hundreds of plants in a day for breeding programs by using only a small amount of (about 10 mg) leaf, root or endosperm tissues, and facilitate the rapid evaluation of primary results. In next stage the primary plants or embryonic sections may be cultivated for subsequent characterization of next generation. In breeding programs for quality improvement, if selection is based on a marker probing

only a single allele of Dx2 or Dx5 (assuming Dx2 is completely linked with Dy12 and Dx5 with Dy10) it would lead to the selection of inappropriate allele combination. Thus, simultaneous selection for both Dx2+Dx12 or Dx5+Dy10 alleles is a critical factor in breeding for quality. In addition to the selection for both alleles, PCR based discrimination system, hampers mistakes resulting from inappropriate allele combinations such as Dx2+Dy10 and Dx5+Dy12 that correspond to low quality. When the alleles of heavy glutenin subunits of commercial cultivars were compared with those of gene bank lines by SDS-PAGE system, it was observed that the Glu-A1 genome of gene bank lines to have a high frequency of the novel allele, which is considered as an unsatisfactory attribute. Alternatively, the Glu-B1 genome of lines revealed a high amount of 7+8 allele which is potentially a valuable resource for transferring their genes to commercial cultivars, which is considered a pronounced advantage for gene bank lines. Finally, it was illustrated that in Glu-D1 genome the level of the worthy 10+5 allele is less than that of commercial cultivars.

p-301
COLOUR STABILITY OF BLACK CARROT ANTHOCYANINS IN VARIOUS FRUIT JUICES AND NECTARS

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Consumer concern over the safety of synthetic food colors has increased the demand for natural food coloring materials. Anthocyanins are the best known natural red pigments used in foods. Fruit juices (apple, grape, and orange) and nectars (peach and pineapple) were colored with black carrot juice and stability of black carrot anthocyanins in these matrices was studied during heating at 70, 80 and 90 ºC and storage at 4 and 37 ºC. During heating, black carrot anthocyanins in apple and grape juices showed higher stability than those in citrus juices at 70 and 80 ºC after 600 min. High stability was also obtained for the anthocyanins in peach nectar at these temperatures. Black carrot anthocyanins were the least stable in orange juice during both heating and storage. During storage, degradation of anthocyanins was very fast at 37 ºC, especially in pineapple nectar. Increase in stability of black carrot anthocyanins was found at 4 ºC after 180 days in all samples.

p-302
PURIFICATION AND ISOLATION OF ANTHOCYANIN PIGMENTS IN BLACK CARROT AND RED ONION

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Anthocyanins are widely distributed in nature in various plant species. They are mainly found in fruits and vegetables. They are valuable coloring agent for food and pharmaceutical preparations. Black carrot (Daucus carota L.) and red onion (Allium cepa L.) anthocyanins were extracted from frozen roots and bulbes at -18˚C, with methanol containing 0.1% HCl (37%v/v). The crude extracts passed through a cation exchange resin column. The eluates were combined and concentrated by rotary evaporator at 34˚C. The final purification was achieved by preparative thin-layer chromatography (TLC) on micro-crystalline cellulose using BunOH-HCl (1:1 v/v) solvent. The isolated bands were dissolved in 0.01% HCl in methanol, and the solvent was concentrated under vacuum. Three pigments were identified in black carrots and four in onions using TLC and UV-visible spectrophotometry.

p-303
DIFFERENTIAL DNA DAMAGE INDUCED IN THE ROOT CELLS OF ALLIUM CEPA SEEDS GERMINATED IN THE HIGH BACKGROUND RADIATION AREAS OF RAMSAR, IRAN

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Assessment of the genetic consequences of the nuclear pollution is one of the most important problems. We have checked out if the plants can serve as in situ monitors for environmental genotoxins. We have used the alkaline comet assay as a method for detecting induced DNA damage in Allium cepa to estimate the impact of high levels of natural radiation in the soils of inhabited zones of Ramsar. The average specific activity of natural radionuclides measured in the soil samples was for 226Ra=12766 Bq kg-1, for 232Th=31 Bq kg-1 and for 40K=611 Bq kg-1. The average specific activity of natural radionuclides measured in the soil samples for 226Ra was 12766 Bq kg-1 whereas in the control soils was in the range of 34 to 60 Bq kg-1. A dose-dependent increase was found in the DNA damage in nuclei of the root cells of Allium cepa seeds germinated in the soil of HBRA. Also, a strong significant correlation of 226Ra specific activity of soil samples with the DNA damage in the roots of A. cepa was observed, (r=0.91). The results showed high genotoxicity of radioactively polluted soils and confirmed the efficiency of the A. cepa comet assay as an ecological and genetic risk assessment in the HBRA.

p-304
STIMULATION OF HUMORAL IMMUNE FUNCTION ACTIVITY BY THE HYDROALCOHOLIC ROOT EXTRACT OF ARTEMISIA ABSINTHIUM LINN IN MICE

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The plant of Artemisia absinthium is a member of the great family of Composite, which is used in the Iranian traditional and folklore medicines for its medicinal actions, as tonic, stomachic, febrifuge and anthelmintic. In this study we have evaluated humoral immunomodulatory effects of the hydro-alcoholic root extracts of this plant, in a mouse model. The fresh roots of A. absinthium were collected from Tehran district during the winter of 2006 and then authenticated. They were shade-dried, powdered and extracted by maceration in 70% methanol and water. The extract was stored in a refrigerator. The mice were divided into 3 groups (n=8). Group I (Control) was given normal saline (0.3 ml/mouse, IP) for 7 days, and Group II and III were given 100 and 500 mg/kg of A. absinthium extract (IP) for 7 days, respectively. The animals were immunized by injecting 0.1 ml of SRBCs suspension, containing 2X 10^7 cells (IP) on day 0. Blood samples were collected in microcentrifuge tubes from individual animals of all the groups by cardiac puncture on day 8. The blood samples were centrifuged and their sera separated. Antibody levels were determined by the haemagglutination technique. Briefly, equal volumes of 50 µl individual serum samples of each group were pooled. Serial two-fold dilutions of pooled serum samples were made in 50 µl volumes of U-shape microtitration plates. To this 50 µl of 1% suspension of SRBC was added. After mixing, the plates were incubated at 37ºC for 30 min to 1 h and examined for haemagglutination under the microscope (button formation). The reciprocal of highest dilution, just before the button formation, was observed and titer values were calculated. The serial dilutions used, were 1/2, 1/4 …, 1/1024. As a thymus-related antibody, the sheep red blood cell (SRBC) was used, which was prepared after washing the sheep red blood cells, three-times in the 0.9% normal saline. The titration results were as follows: in the Control group, all results were negative (100%), and the agglutinations in the groups II and III, occurred in the dilutions of 1/64 and 1/256, respectively. The results showed that the extract of A. absinthium has increased the humoral immune response, and its effect was dose-dependent.

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THE IMMUNOSTIMULATORY EFFECTS OF THE ARTEMISIA ABSINTHIUM (LINNAEUS, 1753) EXTRACT ON THE CELLULAR IMMUNE FUNCTION ACTIVITY IN MICE

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The genus of Artemisia is a member of the great family of Compositae. The flower, branches and root extracts of the plant have been used as a nervine tonic, particularly helpful against the falling sickness and for flatulence in Iranian traditional medicine. It also seems a good remedy as an antipyretic. We have evaluated cellular immunomodulatory effects of the hydro-alcoholic extract of the root of this plant in the mouse model. The fresh roots of A. absinthium were collected from Tehran in Winter 2006. After authentic identification, the roots were shade-dried, and the powder yielded. After 10-12 day storage, the hydro-alcoholic extract was prepared using maceration method in water and 70% methanol. The mice were divided into 3 groups (n=8). Group I (Control) was given normal saline (0.3 ml/mouse, IP) for 7 days, group II and III were given 100 and 500 mg/kg A. absinthium extract IP for 7 days, respectively. Delayed type hypersensitivity test (DTH) was used for evaluation of cellular immunostimulatory effects. In the DTH test, on day 8, the thickness of the right hind footpad was measured using a Vernier calliper. The mice were then challenged by injection of 2 X 10^7sub SRBCs in the right hind footpad, after the previous challenge of the same amount of SRBC, subcutaneously beneath the abdominal skin. The footpad thickness was measured again after 24, 48 and 72 h of the last challenge. The difference between the pre- and post challenge footpad thickness, expressed in mm, was taken as a measure of the DTH response. There was statistically significant difference between the paw edemas from the control and treated groups. But, there was no significant difference between the effects of the two doses of the extract. The findings suggest that A. absinthium has the potential for new therapeutic applications in the future.

p-306

ISATIS SPECIES AS ANTI-LEUKEMIA MEDICINAL PLANTS

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The genus Isatis belonging to the Brassicaceae family is indigenous to India and China. Studies have shown the anti leukemic effect of several species of this genus including Isatis tinctoria. This effect thought to be due to the presence of indoles such as indirubin that are compounds with cell proliferation inhibitory properties. Indirubin is thought to inhibit DNA replication in neoplastic cells without causing significant marrow suppression. Indirubin competes with ATP for binding to catalytic site of cyclin-dependent kinases and block cell proliferation in the late-G1 and G2/M phases of the cell cycle and also inhibits the assembly of microtubules. Several species of Istatis genus are present in Iran, among them is I. campylocarpa which grows in the north of Fars province. The alcoholic and aqueous extracts of the root, leaves and stem of the plant were investigated for possible anti leukemia effects. The anti proliferatory effects of the extracts on two leukemia cell lines including Jurkat and K562 were studied using colorimetric assay. Results obtained indicated that concentrations of 1 to 200 µg/ml of all extracts inhibited the proliferation of the cells. The maximum effect was seen

for aqueous root extract on the Jurkat cells (IC50 10.2 µg/ml). Generally the effect of the extracts on the Jurkat cells was more than K562 cells indicating more sensitivity of lymphocytic cells than myeloid ones.

p-307
EVALUATION OF FLAVONOID COMPOUNDS AMONG WILD SPECIES OF WHEAT IN IRAN

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Flavonoids are important secondary metabolites, which can be used in the evaluation of biodiversity in plant species. So far the flavonoid compounds in wild diploid wheat (Triticum L.; Family Poaceae) species have not been studied. In this study the variability and detection of flavonoid compounds among 25 accessions of wild wheat species such as Triticum boeoticum Boiss., T. thaoudar Reuth., T. monococcum L. and T. urartu Tum. ex Gandil. were shown in different region of Iran. In order to study the variability and purification of flavonoid compounds in each accession, methanol extracts on polyamide Thin Layer Chromatography (TLC; one and two-dimensional maps), with Natural Product (NP) were used to display the flavonoid spots. In order to purify the flavonoid compounds, Sephadex column was used and identification of these compounds was performed based on their UV spectrum at 200-500 nm. The results of this study showed that T. boeoticum accessions have flavonoid compounds such as 2-hydroxychalcone, 3',4',7-trihydroxyflavone 7-O-rhamnoglucoside, 2',4-dihydroxychalcone, fustin 3-O-glucoside, T. thaoudar accessions have 2-hydroxychalcone, 2,2'-dihydroxychalcone, flavone, T. monococcum accessions have flavone, baptigenin, sciadopitysin, 3',4',7-trihydroxyflavone 7-O-rhamnoglucoside and T. urartu accessions have pseudobaptisin, 2-hydroxyl-4'methoxychalcone and flavone. The fractions of flavonoid compounds in each accession possess bathochromic shift which can display the o-diOH in A and B-ring, 7-hydroxylation and 3'-oxygenation. Noticeably, flavone, 2-hydroxychalcone, 3',4',7-trihydroxyflavone 7-O-rhamnoglucoside are the compounds which are similar in some of these wild species. Based on the results of this study T. monococcum and T. boeoticum accessions have the highest variability in their compounds. The percentage of variability in each compound was related to the patterns of oxidation. Finally, the wild diploid wheat species have the simplest flavonoid compounds.

p-308
ANTIOXIDANT PROPERTIES OF CRUDE EXTRACT OF NASTURTIUM OFFICINALIS AN IN VITRO EVALUATION

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The protective effects of fruits and vegetables against chronic diseases have been attributed to the antioxidant properties of some secondary metabolites present in these foods. Nasturtium officinalis (watercress), belonging to Brassicaceae family, has been used as a home remedy by the people of south eastern (SE) region of Iran as a cardioprotective agent. Since this activity may be correlated with the presence of antioxidants, the antioxidant potency of the leaf crude extract was investigated, employing various established in vitro systems, such as the ferric reducing antioxidant power (FRAP) and 2, 2'azinobis 3-ethylbenzothiazoline-6-sulfonate (ABTS+) assays, 1,1-diphenyl-2-picrylhydrazyl (DPPH) /nitric oxide radical scavenging, and iron chelating activity. In the above assays, crude extract of N. officinalis showed antioxidant potential, which was correlated to its total phenolic and flavonoid content. The result of this investigation may show that N. officinalis, as a natural source of antioxidant compounds, may be of use in prevention of free radical-related diseases.

p-309
THE EFFECT OF CHRONIC ORAL ADMINISTRATION OF NIGELLA SATIVUM ON LDL- AND HDL-CHOLESTEROL LEVEL IN DIABETIC RATS

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There are some reports on anti-diabetic effect of black seed in Islamic and traditional medicine. Therefore, hypoglycemic and hypolipidemic effect of this medicinal plant was investigated in an experimental model of insulin-dependent diabetes mellitus. For this purpose, male Wistar rats (n=42) were randomly divided into 4 groups including control, black seed-treated control, diabetic, and black seed-treated diabetic. For induction of diabetes, streptozotocin (STZ, 60 mg/Kg; I.P.) was used at a single dose. A serum glucose level higher than 250 mg/dl was considered as diabetic state. The treatment groups received oral administration of black seed-mixed pelleted food (6.25%) for two months. Serum glucose levels in diabetic group increased at 4 and 8 weeks after the experiment as compared to data one week before the study and Marrubium vulgare treatment of diabetic rats did not have any significant effect. In addition, level of LDL- and HDL-cholesterol increased and decreased in diabetic rats respectively, and black seed treatment significantly reversed this condition. In conclusion, the results demonstrated that oral chronic administration of Nigella sativum could significantly reduce some lipid abnormalities in diabetes and this may minimize some diabetic complications, especially its vascular abnormalities.

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THE EFFECT OF CORDIA MYXA AND ZIZIPHUS SPINA-CHRISTII EXTRACT ON BLOOD GLUCOSE IN RATS

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Traditionally the fruit of Cordia myxa is used for lung and intestine disorders. The objective of the study was to evaluate the effect of the fruit extract of Cordia myxa; and Ziziphus spina-christii on blood glucose in diabetic rats. The hydroalcoholic extract of these fruits was prepared by maceration method. Diabetes mellitus was produced by injection of streptozocin intrapritoneally in 3 groups (n=6 each) of rats. Group1 received oral glibenclamide, group 2 received extract of Cordia myxa and group 3 received extract of Ziziphus spina-christii. Before and 1, 3 and 24 hours after drug administration, the blood glucose of rats was measured by glucometer. The Cordia myxa and Ziziphus spina-christii had similar effects to glibenclamide on lowering blood glucose after 1 and 3 hours after drug administration, but after 24 hours the blood glucose was increased in rats which received these extracts.

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COMPARISON OF THE EFFICACY OF LOVASTATIN AND DILL SEED EXTRACT IN THE REDUCTION OF SERUM CHOLESTEROL IN DIET INDUCED HYPERCHOLESTEROLEMIC RATS

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Introduction: Serum lipid disorders are among important risk factors in development of coronary artery atherosclerosis. The aim of this study was to compare the effects of lovastatin and a dill seed extract on plasma lipids. Methods: Thirty two male Wistar rats (250±30 g) were included and maintained at 23±1 ºC. The animals were divided into 4 groups of 8 each. Groups I and II were given normal and high cholesterol diet respectively, while groups III and IV were given high cholesterol diet along with either lovastatin or dill seed extract for a period of 3 weeks, respectively. At the end of study, blood samples were taken and plasma lipids levels were determined. Results: The results showed that dill seed extract reduced serum levels of TC (from 214.2 to 107.3 mg/dl, p=0.001) and LDL-C (from 149.2 to 37.4 mg/dl, p=0.001) more significantly than lovastatin (from 214.2 to 124.37mg/dl, p=0.001) and LDL-C (frrom 149.2 to 48.13 mg/dl, p=0.001). There was no significant difference in serum triglycerides reduction upon treatment with either dill seed extract or lovastatin. Conclusion: The results of this study indicated that dill seed extract has a lowering effect on the serum lipid levels of diet induced hyprlipidemic rats, and it is more effective in lowering serum total and LDL cholesterol levels.

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EFFECT OF THE PERSIAN WALNUT OIL EXTRACT ON THE LIPID PROFILE IN AN ANIMAL MODEL

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Backgrond: Experimental studies have shown that walnuts (Juglan regia) decrease the risk of coronary heart disease (CHD). Walnut decreases the level of atherogenic lipids such as TG, LDL-C and VLDL-C. Its main effect is induced via ω3-poly unsaturated fatty acids. Walnuts are rich source of these fatty acids, especially alfa -linolenic acid (C18:3; 9, 12, 15).Methods: We assigned 20 hypercholesterolemic male rats (200-250g) to four groups, and fed with four dietary concentrations of the oil extract of Persian walnut (w/w) as a supplement: control group (0%), and experimental groups of 5% (1g oil extract/1g weight /1 day), 7.5%(1.5 g oil extract/1g weight /1 day) ,10% (2 g oil extract/1 g weight/1 day) for eight weeks.Results: There was a positive effect on decreasing the serum concentrations of TG (14%), TC (7.6%), LDL (11%), and VLDL (12%) with increasing consumption of the oil extract of Persian walnut. Conclusion: the findings suggest that walnuts may be used as a dietary supplement to prevent CHD.

p-313
MOLECULAR ANALYSIS OF SALT TOLERANCE IN HALOCNEMUM STROBILACEUM

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Salinity as an environmental stress considered to be the major factor affecting plant growth and distribution in salt marshes. A widely used approach to unravel plant salt tolerance mechanisms has been used to identify genes whose activity is regulated by salt stress. In the present study, we focused on proteins that might be associated with salt tolerance in Halocnemum strobilaceum, one of the most halotolerant species in Eurasia, and to clone their corresponding genes. The Plants and soil samples were collected from three different zones in a salt marsh in the southwest of Iran. The mean salinity was evaluated by measuring electrical conductivity of each soil sample. Total proteins were extracted and analyzed with SDS-PAGE. Comparing the plant protein expression pattern, led to identification of a protein with molecular mass of about 58 kDa, which was absent in the plant related to the zone with less salinity. This conclusion is identical with the assumption that salt regulated genes likely function in more halo-tolerant organisms.

p-314
EFFECT OF ECHINACEA PURPUREA ON CCL4 – INDUCED HEPATOTOXICITY IN RATS

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Echinacea purpurea extract has beneficial antioxidant and immunomodulatory effects and is used in the treatment of influenza and common cold. In this study, we evaluated its prophylactic effect on carbon tetrachloride-induced

hepatotoxicity in rats. The female rats (with mean weight of 200 g) were divided in 3 groups (n=6 each) and were administrated daily for 5 days. Group1 received normal saline (control), group 2 received CCl4, and group 3 received CCl4 + Echinacea purpurea hydroalcoholic extract. After 5 days, serum samples were withdrawn and used for the measurement of serum levels of ALT, AST and ALP. Then, Liver was isolated and sectioned and colored by Heamatoxyline and Eosin and examined by light microscope. The mean of ALT was 34.8, 286.5and 538.6 IU/L in group 1 to 3, respectively. The mean from group 3 was significantly higher than others. The mean of AST was 156.2, 486, 255.8 and 820.8 IU/L in group 1 to 3, respectively. The mean from group 3 was significantly higher than others. The mean of ALP was 398.6, 476.25, 569.2 and 518.8 IU/L in group 1 to 3, respectively. Echinacea purpurea extract increased serum levels of this enzyme. Liver tissue in group 1 had normal hepatocytes in histopathological examination, but fatty change and necrosis of hepatocytes along with lymphocyte proliferation in portal space was observed in group 2. Fatty change was not observed in the liver of rats in group 3. Thus, Echinacea purpurea has antioxidant and immunomodulatory effect and can prevent CCl4-induced histopathological changes in liver, although it increased enzyme release from hepatocytes.

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PURIFICATION OF LIPID TRANSFER PROTEIN 2 (LTP2) FROM RICE PADDY

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Plant non-specific lipid transfer proteins (nsLTPs) are small basic proteins, which transport phospholipids between membranes and are subdivided into two families, nsLTP1 and nsLTP2. In order to purify nsLTP2, rice paddy flour was stirred in sulfuric acid (0.05 M) for 4 h and centrifuged to remove particulate matter. The pH of the supernatant was adjusted to 8.00 and stood at 4°C for 12 h. The precipitate was removed by centrifugation and the final solution, with a rate of 12 ml/h, was loaded onto a CM-Sepharose column, which has been previously equilibrated with 0.05 M Tris-HCl buffer. Bound proteins were separated by a linear gradient of 0 to 0.5 M NaCl. After dialysis, separated protein solution was loaded onto a Phenyl-Sepharose column previously equilibrated with Tris-HCl,,0.05 M; ammonium sulfate, 1.5 M; EDTA, 0.005 M; and NaHSO3, 0.3% at pH 8.4. Tris-Tricin SDS-PAGE was performed for monitoring the purification process. SDS-PAGE of separated proteins from ion-exchange column showed multiple bands. Further purification using hydrophobic column resulted in the appearance of a single band at approximately 7 kDa with 80-90% purity. In order to confirm the presence of hydrophobic surface on nsLTP2, emission changes during binding of 1-Anilino-8-Naphthalene Sulfonate (ANS) to this protein, was studied. Extrinsic fluorescence results has shown that ANS emission is increased and shifted to shorter wave lengths due to binding to the hydrophobic surface of the protein. The results showed that use of ion-exchange along with hydrophobic columns under the above conditions may be a convenient method for nsLTP2 purification.

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THE INTERACTION OF COPPER AND MANGANESE ON GROWTH AND PHOTOSYNTHESIS OF TOMATO LYCOPERSICUM ESCULENTUM, MILL.CV.URBANA VF

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The interactions between copper and manganese on growth and photosynthesis of tomato plants (Lycopersicum esculentum , Mill.cv.Urbana.VF) were studied in pots filled with sand. The plants were treated with Hogland nutrient solution (control), and Hogland solutions containing 30, or 60 µM CuSO4, 30 µM $MnCl_2$, 30 µM $CuSO_4$+30 µM $MnCl_2$ and 60 µM $CuSO_4$+30 µM $MnCl_2$. In 60µM $CuSO_4$, root and leaf protein contents were decreased, while peroxidase (POD) activity of root and leaf were increased, but in roots it was more severe than that of leaves. Copper and manganese toxicities increased soluble and insoluble sugars accumulation of the leaves. The addition of $MnCl_2$ to nutrient solution containing $CuSO_4$ increased reducing or increasing effects on biochemical parameters. In all cases, application of both $MnCl_2$ and $CuSO_4$ resulted in an increase of harmfull effects of $CuSO_4$ on above-mentioned parameters. Since leaf expansion declined, leaves became a weak sink and this might account for the observed accumulation of carbohydrates in leaves. The significant accumulation of starch and sucrose did not occur in roots and seemed to be confined to leaves. This accumulation could induce a feedback inhibition of photosynthesis. Both Cu and Mn in excess affect negatively nitrogen and protein metabolism. Oxidative stress under Cu and Mn toxicity was most probably the consequence of depletion in protein contents. Copper is a transition metal that participates in redox reactions. When in excess, Cu causes over-production of oxy radicals, which is believed to be its primary toxic effects in plant cells. An increase in ROS production was also evidenced by the enhanced POD activity.

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ENHANCEMENT OF COLOR INTENSITY BY PRE-FERMENTATION ADDITION OF COPIGMENTS IN RED CABBAGE JUICE AT THE SAME PH

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Copigments are colorless substances which can form colored cluster with colorless forms of anthocyanins. This copigmentation is widespread in nature. The color changes in fruit, vegetables and flowers may be caused by these reactions between anthocyanins and various organic compounds present in higher plants. The copigmentation equilibrium could be written: Free anthocyanins + copigmentation cofactors = copigmented anthocyanins. The copigments selected for the study included: Catechin, Chlorogenic acid and Gallic acid.

Five levels of copigment concentration (0, 120, 240, 480, 960 mg/l) were examined. The copigmentation effect increased with the increase of copigment concentration. The suitable pH for copigmentation complex was pH 3.5. Catechin was predominat among copigments. The color of anthocyanins can be stabilized and enhanced by the addition of copigments to the red cabbage juice. This addition induced changes in bathochromic shifts and hyperchromic effects that manifest copigmentation of anthocyanin in the buffer solutions.

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THE EFFECT OF TRIBULUS TERRESTRIS ON STRYCHNINE-INDUCED CONVULSION IN RATS

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Tribulus terrestris (caltrops) is traditionally used for diuretic and antistone effect. In this study, its anticonvulsive effect was evaluated in rats. Hydro-ethnanolic extract of Tribulus was obtained by maceration method. Experiment was done in 4 groups of rats as follows: Strychnine was administered subcutaneously at 3 mg/kg in all rats. In group 2, phenobarbital was given 20 min before strychnine administration. Extract of Tribullus was intraperitoneally injected at 250 and 500 mg/kg in group 3 and 4, respectively. The rats were monitored after strychnine administration for 30 min. Duration and number of tonic and clonic convulsions were recorded in monitoring time. Convulsions were significantly decreased by extract of Tribullus. Thus, by this study, it was suggested that Tribulus terrestris can have anti-epileptic effect in strychnine -induced convulsion in rats.

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IN VITRO ANTISPASMODIC COMPOUNDS OF THE POLLEN EXTRACT OBTAINED FROMACHILLEA WILHELMSII

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Antispasmodic activity of methanolic extract of pollen obtained from Achillea wilhelmsii on frog cardiac muscle and isolatedduodenum of guinea pigs were studied. Mature methanolic extract of pollen was compared with immature extract. In order to determine the phytochemical basis for the antispasmodic activity, flavonoid compounds including quercetin, rutin, kampferol and apigenin were extracted from mature and immature pollens. The results indicate that mature pollen methanolic extract have significant expansion ratio to immature pollen methanolic extract.

p-320
IDENTIFICATION OF ARABIDOPSIS THALIANA AT1G05340 (BPR1) INTERACTING PROTEINS IN YEAST

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The study conducted aimed to identify A. thaliana BPR1 (Botrytis and Pseudomonas phaseolicola susceptibility and Root length 1) interacting proteins. Clontech's matchmaker Lex A yeast two hybrid system was used for detecting BPR1-protein interaction in yeast strain EGY 48. This assay is based on the fact that many eukaryotic transcription regulators are composed of physically separable and functionally independent domains. Such regulators contain a DNA binding domain (DNA-BD) that bind to a specific promoter sequence and an activation domain (AD) which directs the RNA polymerase II complex to transcribe the gene downstream of the DNA binding site. The DNA–BD is provided by the entire prokaryotic lexA protein and the DNA-activation domain is contained on an 88 residue acidic E.coli peptide (B42). 3 different vectors used in this system were: p8op-lacZ carrying the lacZ reporter gene with URA3 yeast selection marker, plexA carrying the bait protein with HIS 3 selection marker and pB42AD carrying the cDNA library with TRP1 selection marker. An interaction between the target protein from plexA and a library encoded prey protein from pB42 AD creates a novel transcriptional activator. The BPR1 coding sequence was fused in frame to the LexA DNA-BD and the plasmid was transformed into EGY48+p8oplacZ. Transformants were selected by growth on appropriate double selective media. Expression of the fusion protein was positively tested by Western blot analysis. Auto-activation was excluded by growth on media lacking leucine and also X-Gal color tests. Further, an Arabidopsis cDNA library was transformed into EGY48+p8oplacZ+plexA-BPR1 and approximately 500,000 potential interactions were tested. We found 200 blue colonies indicating many interactions of the BPR1 protein. Further growth tests with increased stringency reduced the numbers of blue colonies to 2. Reisolation of one positive plasmid indicates that the BPR1 protein may interact with CKL2 (CASEIN KINASE l-LIKE 2 (AT5G57015). Retransformation of the pB42-AD plasmid+CLK2 into EGY48+p8oplacZ+plexA-BPR1 confirmed our initial screening result. Further in planta assays are required to test the potential interaction between BPR1 and CLK2.

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DROUGHT STRESS AND ASCORBIC ACID-INDUCED CHANGES OF APX ENZYME IN THE CALLUS OF BEAN: AN IN GEL ENZYME ACTIVITY ASSAY

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To investigate the APX enzyme, drought stress (different percents of PEG 4000) and ascorbic acid-induced changes of APX was examined in the calli of hypocotyle of bean (Phaseolus vulgaris) seedling. Therefore, in each one of PEG percent (0, 2%, 4%, 6%, 8% and 10% PEG treatment), the effect of ascorbic acid (0, 50, 100, 150 and 200 µM) was studied. In order to, analyze the change of APX against drought stress and ascorbate-induced changes, foliar extract was subjected to native PAGE. Extraction of calli fulfilled 12-14 days after calus germination (calli was removed after12-14

days from culture medium). Drought stress preferentially enhanced the activities of APX especially in 8% and 10% PEG. Expression of APX was preferentially enhanced by increase in PEG percent until 6% PEG in medium. Ascorbic acid enhanced APX activity in each one of PEG percents until 100µMAA that were significant. Also, ascorbate increased the expression of APX in 50 µMAA in 2%, 4% and 6% PEG. Expression of APX in 8% and 10% PEG treated with or without ascorbic acid did not different. These results suggest that drought stress activates the APX enzyme that is in Asada-Halliwell-Foyer. Ascorbic acid enhanced enzyme activity and expression proportionally in each one of PEG treatments. Also, this compound increased mRNA related to synthesis of APX in plant cells under stress.

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INTERACTION OF COPPER, ASCORBATE AND GIBBERELLIN ON PEROXIDASE AND CATALASE ACTIVITY IN TWO CULTIVARS OF RAPE SEED (BRASSICA NAPUS L)

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Brassica napus a member of "Cruciferea" Family and of "Brassica" Genus is a rape seed (annual Canola). This agriculture plant has economical importance and is used as industrial and food oil. Seeds of Brassica were cultured in hydroponic environment. Roots and shoots were used for evaluation of different treatments: Cu, Cu and AsA, Cu and GA3 and Cu and AsA & GA3. In this approach the effect of different amount of Cu (5, 15, 25 µmol/lit), AsA (0.5mmol/lit) and GA3 (0.05mmol/lit) on peroxidase and catalase activity in two cultivars of Brassica napus (Hyola 401 and RGS) was investigated. Antioxidant resistance system such as ascorbate, peroxidase and catalase can provide a strategy to enhance stress tolerance. Enzymatic activity of catalase and peroxidase was determined spectrophotometricallyy: (absorption /min/mg protein/fresh weight). Measuring of peroxidase activity performed with Koroi (1989) method and catalase with Chance B (1995) method. 1. In all "Cu treated" plants, enzymatic activity of peroxidase and catalase in shoots and roots was increased. (1-a) This increment in root was more significant than shoots. (1-b) This increment in Hyola was more prominent than RGS variant. 2. In Cu and GA3 treatment enzymatic activity increased. 3. In Cu and AsA treatment enzymatic activity decreased. 4. In Cu and AsA and GA3 "treatment enzymatic activity decreased, but not to level of previous treatment. Generally, it can be said that the "Cu" tolerance of Hyola variety is more than RGS.

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FIBRINOLYTIC EFFECTS OF SOME CURRENT FOOD SAUCES MEASURED BY A FLUORIMETRIC METHOD

GHOLAMALI NADERY, SEDIGHE ASGARY, JAFARIAN, SHAHY, NIKOO NEJABAT, MARYAM KELARDASHT

In disturbances of haemostatic systems such as abnormal clots in vessels and thromboambolia., streptokinase, urokinase and tPA are used for emergency treatment of thromboambolia. Despite their good effects, they have side effects such as general lytic, allergic state, utricaria and serum disease. The objective was to determine if Zataria multifoflora (z.m), Curcuma spp (c.s), Cinnamon spp (ci.s) and Heracleum persicum (H.P) had any fibrinolytic effect. Polyphenolic extract and essential oil of selected plants were prepared by maceration and distillation with water, respectively. The essence was analyzed by G. C. Mass. To evaluate fibrinolytic effect, labeled fibrinogen with FITC was added to plasma, then Ca^{2+} was added to produce labeled clot. The SK (100-1000 Iu/ml), extracts (0.05, 0.5, 5, 50 mg/ml) and essential oils (1/10, 1/100, 1/1000 dilution) alone and in the presence of SK, were added. After 10, 20, 40 and 60 minutes fluorescence was determined (Ex=478, Em= 510). A linear relationship was found between fluorescence and concentration of SK (400-700 Iu/ml). Extract and essential oil of z.m, c.s and ci.s showed significant fibrinolytic effects, but H.P had no significant effect. In the presence of SK, similar results were obtained. The increase in fluorescence for all the plants was not time dependent, except for c.s essence. Essential oil of z.m and c.s and z.m extract showed noticeable fibrinolytic effects. The z.m extract and essential oil increased fibrinolytic effect of SK. It is recommended to study the fibrinolytic effect of their different fractions and look for their other active ingredients.

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CATALYTIC PROPERTIES OF THREE CATALASE ISOENZYMES FROM KOHLRABI (BRASSICA OLERACEA GONGYLODES)

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Kohlrabi (Brassica oleracea gongylodes) is a member of the cabbage family grown for its swollen, turnip-shaped portion of the stem which rests on the ground. Catalase (EC 1.11.1.6) was extracted from kohlrabi bulbs with 0.05 M phosphate buffer, pH 7.0. Kinetic studies for identification of various types of Catalase isoenzymes in Kohlrabi were based on the following criteria (i) variation in activity as a function of pH, (ii) variation in activity as a function of substrate concentration (Km and Vmax), (iii) effect of inhibitors on activity (differential effects of azide and cyanide). Three Catalase isoenzymes were detected in kohlrabi bulb, using hydrogen peroxide as substrate. Their pH optima were 4.5, 6.5 and 10, respectively. Substrate inhibition was found only for the isoenzyme active at pH 4.5. The order of catalytic efficiency (Vmax/Km values) for three isoenzymes of catalase were pH 6.5 > pH 4.5 > pH 10. The effects of two inhibitors, namely azide and cyanide, on kohlrabi catalase isoenzymes showed that, isoenzymes active at pH 4.5 have the highest and the lowest sensitivity to azide and cyanide, respectively.

p-325
ESSENTIAL OIL COMPOSITION OF THE SHASTA DAISY PLANT (CHRYSANTHEMUM MAXIMUM L)

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The Shasta daisy (Chrysanthemum maximum L.) belongs to Asteraceae family and the flowering season of this plant is from May to September. The aim was to study pharmaceutical compounds existing in the essential oil of the plant at different stages such as vegetative, pre-flowering, germination and also assaying compounds present in the flowers and pollen from May to September. The Shasta daisy flowers were collected on first of May and the end of September. Thereafter, they were put into extracting essential oil operations using water distillation method by aid of Clevenger equipment for 3 hours. The obtained essential oils were analyzed by GC and then by GC/Mass spectroscopy.The highest amount of compounds in the vegetative phase were β– Elemene (48.05%) and β – Caryophyllene (18.50%), in the pre-flowering stage, limonene (10.84%) and β–myrcene (10/5%),and in the germination stage, Limonene (12.01%) and Caryophyllene oxide (13.12%). Also, the highest amount of compounds in the flower during spring and summer seasons were Caryophyllene oxide (10.84%), β- Caryophyllene (7.05%), and Limonene (10.07%). The pollen contained β–Elemene (15/5 %), β– Caryophyllene (18.33%) and Caryophyllene oxide (25/5%). The findings indicate that there are a considerable amount of β– Caryophyllene and Caryophyllene oxide which have have anti-caner properties.

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THE PROTEIN CONTENT OF THE POLLEN GRAINS OF SHASTA DAISY PLANT (CHRYSANTHEMUM MAXIMUM L)

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The Shasta daisy (Chrysanthemum maximum L.) is a plant from Asteraceae family whose utilization has found a high degree of popularity in recent years due to ornamental applications. The aim of this study was to investigate the Shasta daisy pollens protein content as well as observation of their allergenic bands. The pollen was extracted in PBS and its protein content was determined by Bradford method and the presence of allergenic proteins was established by SDS-PAGE electrophoresis.. Finally, the potential allergenic property of the pollen extract was followed by Western Immunoblotting test which was applied on guinea pigs. The serum prepared from the pollen extract - sensitized guinea pigs was marked as primary anti-body and anti-IgG1 conjugate specific for guinea pigs, and was chosen under title of secondary anti-body. The tests results indicated that the protein contents of the pollens extract have an OD=0.350. Furthermore, the electrophoretic profile of the pollens extracts showed presence of several protein bands around area between 23KD and 40KD, and specifically 66 KD. As demonstrated in the Western Blot, there, antibody had been produced against 66 KD band in the sensitized guinea pigs that we did applied test repetition of more than 3 times so as to attain better confirmation. The results obtained from different tests and specifically electrophoretic profile and western blot method all demonstrate that there are some reasons indicating allergenic properties of Shasta daisy pollens.

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SUPEROXIDE DISMUTASE ACTIVITY IN NACL STRESS IN SALT-SENSITIVE AND SALT-TOLERANT GENOTYPES OF COLZA (BRASSICA NAPUS L)

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The production of reactive oxygen species (ROS) in salt stress condition causes damage to proteins, lipids, nucleic acids and other sites of cells, this process is a lethal factor for plants. Tolerant plants developed an antioxidant enzymes defense system, which protects them against oxidative damage. Superoxide dismutase (SOD) is a key antioxidant enzyme present in plant cells as a first line of defense against the accumulation of reactive oxygen species. In this study two genotypes of colza, Quantum as a salt tolerant and Fornax as a sensitive, were chosen and germinated in four NaCl concentrations (0, 50, 100 and 10 mM). SOD activity was assayed by monitoring the inhibition of photochemical reduction of Nitro Blue Tetrazolium in roots and shoots of both genotypes in all concentrations. Analyses show that stress induced increases in superoxide dismutase in both genotypes. These salt-induced increases were higher in the salt-tolerant genotype. Increase in SOD activity in roots of tolerant genotype was higher than sensitive genotype. SOD activity decreased in roots of sensitive genotype in 150 mM salt concentration. In shoots, salinity increased SOD activity in both genotypes up to 100 mM concentration, increase in NaCl concentration from 100 to 150 mM caused a decrease in SOD activity in shoots of both genotypes but decrease in SOD activity of salt sensitive genotype (Fornax) was higher than salt tolerant genotype (Quantum). In addition the SOD activity increasing as a result of salt stress was stronger in the salt tolerant genotype compared to the salt sensitive one. Results obtained support the hypothesis the higher efficiency of the antioxidant enzymatic system of Quantum (tolerant) genotype could be considered as one of the factors responsible for its tolerance to salt stress. Therefore, it is suggested that superoxide dismutase activity could be used as a working hypothesis for a biochemical marker for salt tolerance in colza.

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QUANTATIVE AND QUALITATIVE CHANGES IN ESSENTIAL OIL OF ZOSIMIA ABSINTHIFOLIA (VENT).DURING DIFFERENT GROWTH STAGES.

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Zosimia absinthifolia is a permanent herb belonging to Umbelliferae family that grows widely in Iran. The plant materials were collected from Alshtar in North of Lorestan province at three developmental stages including preflowering, flowering and fruiting stages and subjected to hydrodistillation using a Cleavenger– type apparatus for 3 h. Yields of essential oil were 0.42%, 0.65% and 0.8% w/w in

preflowering, flowering and fruiting stages, respectively. Fourty six, 38 and 37 compounds were identified in oils of preflowering, flowering and fruiting stages, respectively. N-octanol, germacrene-D, β-caryophyllene, octyl acetate, caryophyllene oxide, α-pinene and limonene are main components of essential oil of different growth stages.

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ANTIOXIDANT ENZYME ACTIVITIES, PROTEIN CONTENT AND PROTEIN AND ISOZYME PROFILES IN SOME PLANTAGO SPECIES

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The present study aimed at finding out the effect of antioxidant enzymes (SOD, POX, CAT and PPO) on callus formation and regeneratin of shoot and root from leaf derived callus of Plantago psyllium, P.ovta and P.lanceolata Callus was induced from leaf explants excised from 16-day-old seedlings grown on Murashige andSkoog (MS) medium containing 0.5 µM benzyl adenine (BA) and 1.0 µM1-naphtalene acetic acid (NAA). The protein content and enzyme activities were determined by spectrophotometer. SDS-PAGE was used for the determination of protein profiles and PAGE for isozymes. The changes in protein content, enzyme activities, and protein and isozyme profiles in differentiated and non differentiated calluses were found.

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ENZYME ACTIVITY AND GENE EXPRESSION OF PHENYLALANINE AMONIA-LYASE (PAL) IN OCIMUM BASILICUM UNDER DIFFERENT STAGES OF GROWTH

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Phenylalanine amonia-lyase (PAL) is one of the most important enzymes that have a key role in regulation of phenylpropanoid production in plants. It catalyzes the first step of the phenylpropanoids pathway in which L-phenylalanine is deaminated to trans-cinnamic acid. Phenylpropanoid compounds comprise an important part of essential oil of Ocimum basilicum, which is used in traditional Iranian medicine as a culinary herb. There are multiple isoforms of PAL in plants that are encoded by a small multigene family. In this research the rate of gene expression and activity of PAL enzyme was investigated in Ocimum basilicum at different stages of growth. Field experiments took place from 1 July through 15 October and the plants harvested by hand at 5 steps of growth including seedling, first and middle of growth, preflowring and flowering. PAL activity was assayed using spectrophotometer and the level of gene expression was monitored by semi quantitative RT-PCR technique. Plant growth and maturity both affected in the PAL activity. Then we examined the relation of changing in enzyme activity with PAL gene expression in Ocimum basilicum. The detail of our results will be presented in this report.

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COMPARISON OF SPECTROPHOTOMETRIC AND HPLC METHODS FOR THE QUANTIFICATION OF LYCOPENE

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Lycopene is a carotenoid compound with open chain hydrocarbon existing in vegetables and fruits, and is recognized as a strong antioxidant in animal and plant systems. It has received much attention for wide applications in pharmaceuticals, foods and health industries. The main purpose of this research was to optimize the extraction and quantification of lycopene from watermelon. Different ratios of solvents such as hexane, acetone, ethanol, tetrahydrofuran and diisopropyl ether were used to extract the freshly squeezed tissues of watermelon. The extracted lycopene was quantified using UV-visible spectrophotometry and HPLC methods. The best ratio of solvents for Lycopene extraction was found to be hexane/ acetone/ethanol (16:7:7 V/V/V). The amounts of lycopene detected by HPLC and UV- visible spectrophotometry were 2.62 mg/l and 2.32 mg/l, respectively. Briefly, our data showed the potential of HPLC as an accurate and efficient method for quantification of lycopene.

p-332
EXTRACTION OF POLLEN PROTEINS AND ALLERGENICITY OF MATURE AND IMMATURE POLLENS OF ACHILLEA WILHELMSII

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Pollen of Achillea wilhelmsii was collected from area of Esfahan city, Iran. Total protein in mature and immature pollens was extracted using phosphate-buffered saline at pH 7.4 and total proteins were analyzed by Bradford method and electrophoresis on polyacrylamide gel (SDS-PAGE). The allergenicity of mature and immature pollen grains was detected using subcutaneous, skin scratch, conjuctival and inhalation challenge tests. Tests were done on Hartley male guinea pigs. Phosphate-bufferd saline extracts of the pollens were used for subcutaneous test after sterilization by passing through Millipore filters (0.22µ). In treated and control groups the appearance of wheal and flare, their diameter, serum IgE, eosinophilia and white blood cells were compared. Results

showed that: 1) proteins of electrophoresis bands in mature pollens were seen in the range of 14.4, 18.4, 25, 35, 45 and 66.2 KD and for immature pollens about 14.4, 45 and 66.2 KD. 2) mature pollen extract had allergenic properties while immature ones did not.

p-333
ISOZYME STUDIES DURING ROOT FORMATION IN SAFFRON (CROCUS SATIVUS L)

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An attempt was made in the present study to establish a correlation between morphogenic response and isozyme expression, which could be used as a marker system to differentiate the organogenetic potential of explants. Peroxidase, polyphenol oxidase, catalase activities and total protein were measured spectrophotometrically in root forming corm explants of saffron (Crocus sativus L.) at three different concentrations of IBA and NAA on the B5 and MS media. Peroxidase and Lactate dehydrogenase patterns were also studied, and were compared between these treatments and control. Results show that total protein decreased during root formation. In the case of lactate dehydroganase and peroxidase some extra bands appeared during root formation.

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LIGNAN BIOSYNTHESIS IN LINUM PERSICUM IN VITRO CULTURES UNDER DIFFERENT COLOR PLASTIC FILMS

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Lignans are a group of plant phenols with 8, 8′-coupled dimers of coniferyl alcohol or other cinammyl alcohols. This group receives much attention in the field of natural product chemistry ever since the discovery of podophyllotoxin (PTOX). The present interest for this group of natural products is based on their application in the field of pharmacy and nutrition. The attention of pharmacists for lignans in general and PTOX in particular is due to pronounced cytotoxic activity of a number of these compounds. The biosynthesis of lignans in the Linum genus from Linaceae family is documented previously. Aryltetralin lignans like PTOX occur in the section Syllinum. Many experiments follow to establish plant in vitro cultures from different plants not only to biosynthesize PTOX but also other lignans. In these studies shoot cultures of Linum persicum were established on MS (Murashige and Skoog) medium containing kinetin (2mgl-1). After culture establishment; green, red, blue, yellow, gray plastic films in comparison with florescent light (ca 4420 lx) were used to see the effects of different color plastic films on shoot regeneration and lignan biosynthesis. HPLC/ MS and HPLC/ UV-DAD were used to identify lignans and HPLC method was used to measure the amount of lignans accumulating in L. persicum shoot cultures. Results showed that red plastic films have significant ($p \leq 0.001$) effects on PTOX biosynthesis (0.75±0.03 % g/100g DW) and blue and green plastic films cause a significant decrease in PTOX biosynthesis (0.07±0.02 and 0.21±0.03 % g/100g DW, respectively). The comparison of results obtained from shoot morphogenesis and lignan biosynthesis showed that there is a direct relationship between shoot elongation and PTOX biosynthesis.

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EFFECTS OF SALINITY ON GROWTH PARAMETERS, LIPID PEROXIDATION AND ANTIOXIDANT ENZYMES IN THREE ACANTHOPHYLLUM SPECIES OF DIFFERENT PLOIDY LEVELS

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The genus Acanthophyllum C. A. Meyer comprises of 61 species, 33 of which occur in Iran and most of them grow in saline soils. In this study we investigated the effect of salinity on growth parameters, lipid peroxidation and antioxidant enzymes in calli of three Acanthophyllum species with different ploidy levels including A. laxiusculum Shiman-Czeika (diploid species), A. sordidum Bunge ex Boiss. (tetraploid species) and A. glandulosum Bunge ex Boiss. (hexaploid species). Calli of the species were subjected to NaCl stress (50, 100 and 150 mM) for 30 days. Salinity caused a reduction in relative growth rate (RGR) and relative water content (RWC) with a greater reduction in A. laxiusculum. However, salinity stress caused only slight decrease in RGR and RWC of A. glandulosum. Under salinity stress, the diploid and tetraploid species, exhibited a decrease in superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR), with a greater reduction in diploid species. MDA content also increased under salt stress. The hexaploid species A. glandulosum however, exhibited an increase in SOD, CAT, APX and GR activities and virtually unchanged lipid peroxidation under salinity stress condition. According to our results, A. glandulosum showed a better protection mechanism against salinity induced oxidative damage than A. sordidum and A. laxiusculum. With regard to the role of polyploidy in the evolution and speciation of the Acanthophyllum genus, higher ability for salt tolerance observed in A. glandulosum, might have been due to the greater polyploidy level in this species compared to the other ones.

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THE ANTIOXIDANT EFFECT OF SOME PLANTS OF LABIATAE FAMILY

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Damage to cells and tissues by oxidation of poly unsaturated fatty acids(PUFA) is one of the most important risk factors of

many diseases , such as ,atherosclerosis, rheumatiod arthritis, cancer, AIDS and aging. Usually oxidation is due to the increase of oxidant agents (such as, free radicals) or decrease of antioxidants. It has been shown that some flavonoids and polyphenols have antioxidant activity. The aim of this work was to study the antioxidant activity of some plants of Labiateae family. The antioxidant activity of total and polyphenolic extracts of some plants of Labiatae family in three different concentrations (0.05, 0.01 and 0.005 mg/ml) were undertaken on rat hepatocyte membrane for PUFA. This activity was shown by the measurement of formation of MDA and leakage value of SGOT and LDH of Thymus daenensis, Lavandula officinalis, total and polyphenols of Melissa officinalis, Saliva officinalis, and the total extract of Dracocephallum kotschy Boiss. In conclusion, selected plants showed antioxidant activity, and this effect was dose-dependent, but the effective dose varied in different extracts. However, in the low concentration (0.005 mg/ml) total extract of Melissa officinallis was the only extract which had antioxidant activity.

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EXTRACTION AND PURIFICATION OF LYCOPENE FROM TOMATO SAMPLES

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Carotenoids are highly unsaturated hydrocarbons present in all plants, algae and cyanobacteria. Lycopene is the most potent antioxidant among carotenoids as it has the highest singlet oxygen quenching rate of the all the carotenoids found in biological systems. This compound has important applications for use in foods, nutritional supplements and pharmaceuticals. The overall goal of this study was to extraction and purification of lycopene from tomato. Different ratios of solvents such as hexane, acetone, ethanol, tetrahydrofuran and as disopropyl ether were used to extract the freshly squeezed tissues of tomato. The extracted Lycopene was quantified using HPLC methods. Under the best conditions, the ratiosof solvents for Lycopene extraction from tomato was obtained as hexane/ acetone/ethanol (16:7:7 V/V/V). The lycopene concentration detected by HPLC was 2.75 mg/l. This study demonstrated the HPLC can be used as a reliable and simple method for determination of lycopene concentration.

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KINETIC AND STABILITY STUDY OF SUPEROXIDE DISMUTASE IN THE PRESENCE OF SUCROSE AS AN OSMOLYTE

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Increasing levels of active oxygen species (ROS) or free radicals can create an oxidative stress, which is toxic and dangerous for plant cells. In plants, ROS can be omitted by distinct enzymatic defense mechanisms. Superoxide dismutase (SOD) is a metaloenzyme that omits the superoxide radicals and converts them to hydrogen peroxide. This enzyme has three types according to the presence of metal in its active site. These types are iron-containing superoxide dismutase (Fe-SOD), manganese-containing superoxide dismutase (Mn-SOD), copper/zinc-containing superoxide dismutase (Cu/Zn-SOD). We know that salt stress generate an increase in the level of osmolytes to protect cellular osmotic pressure and also make oxidative stress. Therefore, we have considered structural and kinetic effects of sucrose on horse radish superoxide dismutase. Several experimental methods were utilized such as gel electrophoresis to determine molecular weight of the enzyme, UV-visible spectrophotometer to ascertain activity of the enzyme, scanning UV-thermal method to ascertain thermal stability of the protein in the presence of sucrose. The results indicate that in the presence of sucrose the enzyme activity decreases.

p-339
STUDY OF COLLAGEN DEGRADING ACTIVITY OF FICIN AND ACTINIDIN

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Extracts of plants, such as kiwi fruit and fig are known to be effective for meat tenderization. This study investigated collagenolytic activity of Actinidin and Ficin, major cysteine proteases in kiwi fruit and fig, respectively. Actinidin and Ficin were purified by ion exchange chromatography. Collagenolytic activity of these purified proteases were assayed and characterized by electrophoresis in 12% poly acrylamide gel in the presence of SDS. Different amounts of these proteases added to constant amount of collagen type I and incubated in 37 ºC for 1 hour. After incubation, the reaction stopped by heating in boiling water. The results of electrophoresis showed that these two cysteine proteases can degrade collagen effectively in neutral pH.

p-340
HYPOLIPIDEMIC EFFECT OF AQUEOUS EXTRACT OF TRIGONELLA FOENUM-GRAECUM IN DIABETIC RATS

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Introduction: Considering the hypoglycemic and hypolipidemic effect of fenugreek seed and hypoglycemic effect of its leaf, this study was conducted to evaluate the hypolipidemic effect of the latter part. Matreials and Methods: Male Wistar rats were divided into control, extract-treated control, diabetic, and extract-treated diabetic groups. Fenugreek extract was administered at doses of 100 and/or 200 mg/Kg intraperitoneally every other day three days after the induction of diabetes for a period of 2 months. The serum levels of total cholesterol, triglyceride, LDL- and HDL-cholesterol were determined one week before the study and at 4th and 8th weeks after treatment. Results: Serum total choleasterol significantly increased in diabetic rats, and extract

treatment did not change it significantly. In addition, there was no significant increase in triglyceride level in diabetic rats and no significant effects of the extract. On the other hand, LDL- and HDL cholesterol levels significantly increased and decreased in diabetic rats, respectively. The administration of the extract significantly reversed these changes. Conclusion: The results showed that treatment of diabetic rats with aqueous extract of fenugreek could improve the inappropriate changes in LDL- and HDL-cholesterol.

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DEGRADATION OF ALLERGENIC SOYBEAN KUNITZ TRYPSIN INHIBITOR BY FIG CYSTEINE PROTEASE: FICIN

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The soybean Kunitz trypsin inhibitor (SKTI) is a 21.5 kDa allergenic protein that belongs to the family of all antiparallel beta-sheet proteins that are highly resistant to thermal and chemical denaturation. SKTI was purified from defatted soybean meal by affinity chromatography with immobilized trypsin. Ficin was purified from green fig fruit by ion exchange chromatography. SKTI degradation activity of this purified protease was assayed and characterized by electrophoresis in 12% poly acrylamide gel in the presence of SDS. Different amounts of this protease added to constant amount of purified SKTI and incubated at 37^{o} C for 1 hour. After incubation the reaction stopped by heating in boiling water. The results showed that ficin can degrade SKTI effectively in neutral pH.

p-342
PURIFICATION AND THE INHIBITORY EFFECT OF AN ALPHA –AMYLASE INHIBITOR FROM WHEAT

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Alpha-amylase inhibitors found in seeds are drug-design targets for treatment of diabetes and digestion disorders. These inhibitors are also known as sensitizing agents in humans. Plant alpha-amylase inhibitors show great potential as tools to engineer resistance of crop plants against pests. Numerous forms of alpha –amylase inhibitors have been reported. Alpha-amylase inhibitor was extracted from Iranian wheat cultivar (Triticum aestivum v zarrin), precipitated and purified by anion exchange fast protein liquid chromatography. Electrophoresis of purified protein showed a 0.59 relative mobility. Total hydrolytic activity of human salivary and bacillus subtilis alpha –amylase were inhibited 92.01% and 78.99%, respectively by purified alpha- amylase inhibitor.

Structure-Function Relationship

O-343
SEQUENCE AND STRUCTURAL PARAMETERS ENHANCING ADAPTATION OF PROTEINS TO LOW TEMPERATURES

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A systematic analysis compared sequence and structural parameters distributions between 13 pairs of psychrophilic and mesophilic proteins for elucidating the cold adaptation parameters. The results of statistical test (t-test) revealed that helical content, tight turn content, disulfide bonds and hydrogen bonds don't show significant difference between psychrophilic and mesophilic proteins. However, it was demonstrated in this study that a larger proportion of open β-turn in psychrophilic proteins is an effective parameter in specific activity at low temperature. In addition, substitution of amino acids of charged and aliphatic groups with amino acids of tiny and small groups in protein chains, tight turns and α-helices in the direction from mesophilic to psychrophilic proteins is one of the mechanisms of low temperature adaptation. Such sequence and structural parameter differences would help to develop a strategy for designing cold-adapted proteins.

p-344
FFECT OF AGEING TIME ON THE SOL GEL DERIVED NANOSTRUCTURED HYDROXYAPATITE POWDERS AND COATINGS

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Due to the chemical similarity between hydroxyapatite (HA) and mineralized bone of human body tissue, synthetic HA exhibits strong affinity to host hard tissues. To achieve better biocompatibility and excellent mechanical performance of prostheses, HA coating is often fabricated on titanium surfaces. The aim of this work was to evaluate the effect of ageing time on the formation and phase purity of hydroxyapatite (HA) nanostructured powders and coatings. Hydroxyapatite powders and coatings were prepared using a sol–gel method with calcium nitrate and phosphorous pentoxide as starting materials. Different ageing times were employed in order to evaluate the effect of ageing time on the phase purity of the HA powders. Precursor sols were dip-coated onto the cp Ti substrates and, then the plates were aged and dried. Finally samples calcined at 600ºC and 700ºC temperatures. Thermal behavior, phase formation, surface morphology and interfacial coherency were investigated by thermogravitometry analysis (TGA), X-ray diffraction (XRD) and scanning electron microscopy (SEM). It was found that a solution ageing time of at least 24 h was required to achieve monophasic hydroxyapatite nanostructured powders and coatings. Formation of the hydroxyapatite structure of coating was observed at 600°C by X-ray diffraction and a β-tricalcium phosphate phase was formed at 700°C. The effect of firing temperatures on the crystallite size of coatings was not significant. The HA film had a thickness of 15 μm after the

heat treatment at 600°C. SEM observations revealed no delamination and crack at the interfaces of HA/Ti.

p-345
γ-TURN TYPES PREDICTION IN PROTEINS USING THE SUPPORT VECTOR MACHINES

SAMAD JAHANDIDEH, PARVIZ ABDOLMALEKI, EBRAHIM BARZEGARI ASADABADI

Due to the slightly success of protein secondary structure prediction using the various predictor models, different models have been developed for predicting γ-turns in proteins by Kaur and Raghava [2003; A neural-network based method for prediction of γ-turns in proteins from multiple sequence alignment. Protein Science, 12: 923-926]. However, the major limitation of previous methods is inability in predicting γ-turn types. Thus there is a need to predict γ-turn types using an approach, which will be useful in overall tertiary structure prediction. In this work, Support Vector Machines (SVMs), a powerful model is proposed for predicting γ–turn types in proteins. The high rates of prediction accuracy showed that the formation of γ–turn types is evidently correlated with the sequence of a teripeptides, and hence can be approximately predicted based on the sequence information of the teripeptides alone.

p-346
α-CYCLODEXTRIN-ACRYLAMIDE COPOLYMER AS AN EFFICIENT STRIPPING AGENT IN SOLID-PHASE ARTIFICIAL CHAPERONING STRATEGY

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Recently, insoluble cyclodextrin copolymers have been used for the refolding of thermally and/or chemically denatured proteins in artificial chaperone-assisted strategy. To enhance the refolding yield with lower quantities of copolymers, a new copolymer with high cyclodextrin content was synthesized. First, the primary hydroxyl groups were derivatized with reactive allyl-containing groups and then the allyl-cyclodextrin product was copolymerized to obtain an acrylamide-based water-insoluble gel. The ability of the copolymer in the refolding of thermally and/or chemically denatured carbonic anhydrase was tested and experimental variables (e.g. copolymers and the protein contents) were optimized to improve the refolding yields along with depressing the aggregate formation. In addition, comparative studies were accomplished under different ionic strength conditions. Our results (activity and extrinsic fluorescence data) showed that, under the optimal developed refolding environment, the denatured enzyme recovered more than 70% of its initial activity using less than 10 mg of the copolymer/ml refolding solution. In this presentation, we will discuss the significance of these observations.

p-347
THE EFFECT OF MOLECULAR CHAPERONES ON AMYLOID FORMATION IN CROWDED SYSTEMS

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Amyloid fibrils arise from the slow aggregation of intermediately folded protein states. In this study we have compared the kinetics of the protein fibril formation and its prevention (destabilized α-lactalbumin, αs- and k-casein) by α-crystallin in the presence or absence of dextran (68 kDa). These target proteins are very different in their size, structure, organs and properties. Bovine α-lactalbumin, αs- and k-casein form amyloid fibrils at low pH or in a reducing environment. An increase in the thioflavin T fluorescence intensity upon the addition of dextran as a macromolecular crowding agent reveals that the rate and extent of amyloid formation were significantly increased. However, the effect of α-crystallin in preventing fibril formation was significant, although reduced in comparison with the absence of crowding.

p-348
AN INVESTIGATION ON THE STRUCTURE OF CALPROTECTIN IN THE PRESENCE OF ZINC

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Calprotectin is a heterodimeric complex, belongs to S100 protein family with two small (MRP8) and large (MRP14) subunits. It has been found predominantly in cytosolic fraction of neutrophils and contains two calcium binding EF-hand motifs and at least one distinct zinc-binding site in each subunit. Calprotectin inhibits activity of matrix metallo proteinases (MMPs) by sequestration of zinc. Previous studies have shown that calprotectin has growth inhibitory effects against normal fibroblasts that regulate the repair of wound site through cell growth and production of the material covering the intracellular matrix. In this study calprotectin was purified from human neutrophils, using a two-step ion exchange chromatography (Q-sepharose and SP-sepharose). The growth inhibitory activity of purified calprotectin was examined on MOLT4 cell line by MTT assay, Annexin V kit. For structural and thermal analysis, the purified calprotectin was used in the presence of different concentration of Zn. Analysis of Far UV-CD spectra of human calprotectin, demonstrated that Zn affected secondary structures of the protein. The intrinsic fluorescence emission spectrums of the human calprotectin (50μg/ml) in the presence of Zn indicated a significant change in the fluorescence intensity, reflecting conformational changes within the protein. Structural changes of calprotectin upon zinc binding seem to be important in some biological roles of this protein. Our studies demonstrated that the thermal stability of calprotectin in the presence of Zn markedly decreases. These results show that changes in the calprotectin structure in the presence of Zn may prevent its interaction with other proteins especially its receptor.

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DESIGN OF A COMPUTER MODEL LUNG SURFACTANT LIPID FOR EVALUATION OF

EFFECTS OF SOME PETROCHEMICAL SOLVENTS ON DIPALMITOLPHOSPHATIDYLCHOLINE

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Surfactant is the main compound that prevents collapse of the lung. DPPC (Dipalmitolphosphatidylcholine) is the major lipid component of the lung surfactant that lowers surface tension of alveoli. Exposure to some petrochemicals may lead to lung injury, which is a result of changes in the structure of the surfactant. To describe the interactions between DPPC as an important component of the lung surfactant and solvents like Benzene, Toluene, Heptane, Acetone, Chloroform, Ether and Ethanol we have used a model designed by computational methods of Ab initio and Molecular Dynamic. Based on the existing theories these chemical solvents cause lung injury by changing the conformation of DPPC. The effect of these chemical solvents on the conformation of DPPC Head Group leading to its disorder has been investigated and studied by Gaussian 03 and Charmm software. Finally, The validity of the simulated model has been established by the results obtained from NMR investigations. There are a few studies on the effects of pollutants on the structure of lung surfactant, especially DPPC. According to the result we obtained, Ethanol plays the most important role in changing the conformation and lipid disorder. Our findings suggest that ethanol used in industry is one of the risk factors that cause lung damage. The findings can also be useful for detecting dysfunction of DPPC in lung surfactant due to acute or chronic exposures to air Pollutants such as petrochemical organic solvents and habitual consumption of alcohol. Further studies are needed to define the relation between chronic ethanol consumption and lung damage.

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THERMAL UNFOLDING STUDIES OF MOUSE ANTI DIGOXIN MONOCLONAL ANTIBODY BY SPECTROSCOPIC METHODS

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Since recently, immunoglobulinG (IgG) molecules, i.e. antibodies, are widely used as convenient and valuable tools for various immunochemical and biochemical analyses. The availability of these monoclonal antibodies resulted in the development of various diagnostic tests. In this study, mouse monoclonal antibody (IgG1) against digoxin, a cardiac glycoside was structurally investigated. Since immunoglobulin, IgG, shows a strong structure-function relationship, knowledge about stability in various conditions may be helpful in immunochemical applications. The effect of temperature on secondary structure of IgG was investigated by circular dichroism (CD) technique and the temperature scan at 206.5 nm was recorded. Another technique for the study of IgG structure is absorbance and fluorescence spectroscopy. Fluorescence spectroscopy was done with Varian Cary Eclipse spectrofluorometer and all measurements performed in a thermostat cell holder. Protein concentration of 150µg/ml and path length of 1cm was used. Samples were excited at 295nm and emission spectra from 310 to 500nm were recorded. Turbidimetric measurements were carried out using spectrophotometer (Agilent 8453) at the wavelength of 418 nm. Since proteins do not show absorbance at this wavelength, the observed absorbance arises from light scattering. The fluorometery data showed structural changes upon heat treatment. Decreased fluorescence intensity and the red shift both represented the unfolding and exposure of tryptophan residues to the solvent. Tm of IgG established to be 78ºC and denaturation was reversible to some extent. On the other hand turbidimetery measurements revealed no aggregation of IgG upon heat treatment up to 100ºC. Thus structural stability of the above mentioned IgG implies that it can be a useful tool in immunochemical studies and therapeutic applications.

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MOLECULAR MODELING OF BENZOTRIAZOLE AND TRIAZOLE DERIVATIVES AS 6PGDH ENZYME INHIBITORS IN TRYPANOSOMA

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Human African trypanosomiasis (sleeping sickness) is caused by trypanosoma species and is transmitted to human by tsetse flies. 6-Phosphogluconate dehydrogenase (6-PGD) is used as one of the possible potent targets for chemotherapy against Trypanosoma brucei. This enzyme catalyzes the reversible oxidative decarboxylation of 6-phosphogluconate to ribulose 5-phosphate and CO_2 with the concomitant generation of NADPH. Recently, several triphenylmethane derivatives have been reported to have a specific inhibitory effect against 6-PGD of the parasite comparing to that isolated from sheep. In the present study we synthesized some triphenylmethane derivatives. Using Autodoc program, we docked these componds on 6-PGD enzymes (Protein Data Bank) isolated from both T. brucei and sheep. Based on our data, these compounds showed specific inhibitory effects on 6-PGD enzyme isolated from T. brucei rather than that isolated from sheep. Docking study revealed that these synthetic compounds show different binding energies for 6PGDH compare with those shown by the 6PG ligand. Collectively our synthetic compounds might have potential ability to inhibit the 6PGDH enzyme selectively.

p-352
LINEAR DISCRIMINANT ANALYSIS APPLIED TO QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIPS FOR DUAL BINDING SITE INHIBITORS OF ACETYLCHOLINESTERASE

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The most important and well-documented function of acetylcholinesterase (AChE) is the hydrolysis of the neurotransmitter acetylcholine. This enzyme has long been an attractive target for rational drug design and discovery of mechanism based inhibitors for the treatment of Alzheimer's disease (AD), Parkinson's disease and myasthenia gravis. Dual binding site AChE inhibitors as a new generation of anticholinesterase drugs have shown a great inhibitory effect on the enzyme because of their simultaneous interaction with both catalytic and peripheral sites of AChE. In this study, we applied linear discriminant analysis (LDA) as a classifier model to investigate the quantitative structure activity relationships in dual binding site inhibitors of AChE. A database of 24 new dual binding site AChE inhibitors was used to extract and evaluate the most effective structural parameters, which determine the inhibitory potential of compounds. We classified the 24 compounds into two active (IC50<2nM) and inactive (IC50>2nM) groups. Using linear discriminant analysis, we first selected the most effective structural descriptors and then developed a linear model with the ability to classify such compounds into those activity groups based on selected parameters. A 91.7% measure of accuracy in classification of AChE inhibitors was obtained by the proposed linear discriminant model.

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ACTIVITY CLASS PREDICTION OF DUAL BINDING SITE ACETYLCHOLINESTERASE INHIBITORS AS NEW CANDIDATES FOR ALZHEIMER'S DISEASE THERAPY

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Acetylcholinesterase inhibitors are currently the most successful therapeutic agents for patients with Alzheimer's disease (AD). Recently, a new generation of anticholinesterase drugs called dual binding site acetylcholinesterase inhibitors have been synthesized and examined for their enzyme inhibitory activity. Newly synthesized compounds show a great inhibitory effect on the enzyme because of their unique structural pattern, which allows them to interact simultaneously with both catalytic and peripheral sites of acetylcholinesterase. Dual binding site acetylcholinesterase inhibitors have thus been considered as new potent candidates for the treatment of AD. In this study, a database of 24 dual binding site inhibitors was used to extract the most effective structural parameters, which influence the inhibition activity of compounds. The compounds in the database were classified into two defined activity classes based on their inhibition activities. Then, binary logistic regression analysis served on the database both to select effective structural parameters and to build a mathematical model for activity class prediction of cholinesterase inhibitors of the same type. A prediction accuracy of 79.2% was obtained by using the logistic regression model.

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THE CONFORMATIONAL CHANGES IN DNA AND OLIGONUCLEOTIDES DUE TO INTERACTION WITH SAFFRON AND ITS INGREDIENTS

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It is already known that saffron and its ingredients such as crocin, crocetin, picrocrocin and safranal share the innate DNA binding property. Many biological properties of saffron have been reported, among which the anticancer property is the most important. Some anticancer drugs show a direct interaction with DNA, and thus the present study aimed at investigating the effect of saffron and the above mentioned constituents on the structure of DNA and oligonucleotides as a possible mechanism of their anticarcinogenic action. The interaction between four molecular components of Iranian saffron, with calf thymus DNA (ct-DNA), G.C and A.T oligonucleotides were studied in vitro by spectrophotometrical titration and monitoring the circular dichroism (CD) spectral profile. Saffron and its ingredients showed to bind to DNA minor groove. The CD spectroscopy enabled us to identify the types of interactions. The non-intercalative binding mode of saffron and its ingredients with (ct-DNA), oligo (dG-dC)15 and oligo (dA-dT)15 caused transition from the B to the C-DNA and then unstacking of DNA bases at higher concentrations of the molecular components of Iranian saffron which cause the decrease in DNA and oligonucleotides stability. Saffron carotenoids studied here (crocin and crocetin) showed similar affinities for G.C and/or A.T sequences. In conclusion, the results showed that saffron and its ingredients interact with and induce some conformational changes in the DNA structure with the order of potency of crocetin > > crocin >picrocrocin and saffranal.

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EPITOPE MAPPING OF PR81 ANTI-MUC1 MONOCLONAL ANTIBODY FOLLOWING PEPSCAN AND PHAGE DISPLAY TECHNIQUES

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PR81 is an anti-MUC1 monoclonal antibody (mAb) which was generated against human MUC1 mucin that reacted with breast cancerous tissue, MUC1 positive cell line (MCF-7, BT-20 and T-4 7 D) and synthetic peptides including tandem repeat sequence of MUC1. Here, we characterized the binding properties of the PR81 against the tandem repeat of MUC1 by two different epitope mapping techniques, namely, PEPSCAN and Phage-display. Epitope mapping of PR81 mAb by PEPSCAN revealed a minimal consensus binding sequence, PDTRP, which is found as the most important epitope on MUC1 peptide. Using phage-displayed peptide library, we have identified the motif PD (T/S/G) RP as an epitope and the motif AVGLSPDGSRGV as a mimotope recognized by PR81. Results of these two methods showed that the two residues, arginine and aspartic acid, have an important role in antibody binding and threonine can be substituted by either of glycine or serine. These results may be of importance in tailor making antigen used in immunoassay.

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SYNTHESIS, INTESTINAL ABSORPTION AND TOXICITY OF 3-HYDROXYPYRIDINONES IN RATS

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1,2-Dimethyl-3-hydroxypyridinone (deferiprone) is currently used for the treatment of iron-overload in thalassaemic patients. Some toxic effects, however, have been reported in certain patients receiving deferiprone. Synthesis of a less toxic iron chelator is, therefore, imperative. In this study, a less toxic hydroxypyridinone ligand namely 2-methyl-3-hydroxy-5- (dimethyl aminomethyl) pyridinone (HPO) was synthesized. The partition coefficient (Kpart) and the intestinal absorption (IA) of HPO were determined. The HPO ligand was synthesized from maltol and ammonia via a Mannich reaction in a four-step reaction method and its purification was achieved by elemental analysis. The Kpart values of both ligands were measured in 1-octanol / tris buffer (pH 7.4) and their IA were also determined using Everted Gut Sac (EGS) at various concentrations. The results showed that, at the concentration of 60 mg/L and after 40 minutes, the absorption was reached to a maximum for both ligands. The Kpart value of HPO was higher than that of deferiprone. Therefore, a higher IA would be expected for HPO. Nevertheless, the rate and extent of intestinal absorption of HPO were not statistically different from those of deferiprone. It therefore seems that, IA of HPO by EGS depends not only on Kpart value, but also on other factors such as the number of hydrogen bonds between the drug and surrounding molecules. In conclusion, HPO did not exhibit greater IA than that of deferiprone, but was associated with less toxicity.

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STRUCUTRE- FUNCTION STUDY OF HISTONE H1 IN NORMAL AND DIABETIC RATS

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Chronic hyperglycemia increases the non-enzymatic glycation of inter and intracellular proteins in diabetes mellitus. It is well established that the function of a protein depends on its native structure; hence, glycation can induce structural and functional alterations in a variety of proteins and these modifications could be involved in the pathogenesis of some complications of diabetes. Histone H1, a basic protein susceptible to glycation, is characterized as a packing agent of chromatin and general controlling factor of gene expression. We aimed to study the effect of glycation on H1 structure and function. In this study Male Wistar rats were divided into two groups (control & diabetic). One group was injected intraperitoneally with streptozotocin (50 mg/ kg body) and thereafter only rats with blood glucose levels ≥ 270 mg/dl were included in our experiments. Histone H1 was extracted from the liver of normal rats and its secondary structures and function were analyzed by circular dichoroism and UV- spectroscopy, respectively. For the first time histone H1 in glycated form was extracted from the liver of diabetic rats by salt-method extraction. Its structure compared with the normal H1 by CD and its interaction with DNA was studied by precipitation method using UV-spectrophotometry. Findings indicated the conformational and functional changes of Histone H1 in diabetic rats in comparison to normal animals. Therefore due to central role of Histone H1 in chromatin packing and gene expression, some pathogenesis of diabetes mellitus could be attributed to structural changes at the level of H1 and to the interaction of this protein to the DNA.

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IN VIVO AND IN VITRO STUDY OF PROTEIN STRUCTURE-FUNCTION DUE TO HIGH GLC CONCENTRATION AND THE IMPROVEMENT EFFECT OF CHEMICAL CHAPERONES

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Nonenzymatic glycation of proteins induced by hyperglycemia in diabetes affect structure and function of biomacromolecules. The aim of this study was to determine the effects of high-dose and long-term use of different chemical chaperones on the structure and function of proteins in diabetic rats and in the test tube. The streptozotocin-induced diabetic rats were used as a model of typeI diabetes. Normal and diabetic rats were studied for 5 months without or with one of the chemical chaperones (L-Lys, ASA, spermine or BJ-83) added in drinking water. Diabetic rats from different aspects such as induction of heat shock proteins (HSP70) as well as its protective effect on protein structure and function and hence its effect on serum Glc and insulin level, AGE and HbA1c formation, lipid profile, HDL functionality (paraoxonase and LCAT activities) and the status of the antioxidant defense system through FRAP assay were investigated. In addition, the in vitro effect of Glc in the presence and absence of chemical chaperones on the structure of albumin as a protein model was studied by spectroscopic techniques such as fluorometry and circular dichroism (CD). The in vivo data showed that chemical chaperones, with

different degrees, decreased mortality of rats by prevention of protein glycation as revealed by decreased AGE and HbA1c), improvement of lipid profile and HDL functionality (decrease in serum lipids and LDL-c, and an increase in HDL-c, LCAT and PON1 activity), elevation of antioxidant capacity and HSP70 induction. The in vitro study indicated that the conformation of BSA is changed due to glycation and chemical chaperones retain the conformation of this protein in its native form. In conclusion, chemical chaperones studied here showed beneficial effects on the nonenzymatic glycation induced misfolding of proteins that is a consequence of diabetic induction, through different mechanisms.

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POLYAMINES EFFECT ON GLUCOSE-INDUCED LYSOZYME MODIFICATIONS

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Lysozyme (1,4-b-N-acetylmuramidase; EC 3.2.1.17) is a natural antibacterial protein found in saliva, nasal secretions, milk, mucus, serum and in the lysosomes of neutrophils and macrophages. This protein binds specifically to glucose-modified proteins bearing advanced glycation end products (AGEs). Exposure to AGE-modified proteins inhibits the bactericidal and enzymatic activity of lysozyme. Nonenzymatic glycation of proteins, peptides and other macromolecules (the Maillard reaction) has been implicated in diseases such as diabetese mellitus and in the normal processes of aging. The present study examined the effect of glycation and subsequent structural modifications of lysozyme by polyamines. Lysozyme at 10 mg/ml in phosphate-buffered saline was incubated with glucose (50, 100 mM) in a sterile condition at 37 degrees C for 120 days. Structural modifications and the activity of lysozyme were observed by fluorescence measurements, circular dichroism (CD) and UV/VIS spectrophotometry. When lysozyme and glucose were incubated in the presence of spermine (50 μM), more structural changes were observed in comparison to the glucose alone.

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MOLECULAR DYNAMICS SIMULATIONS OF INTRA-MOLECULAR CHAPERONE PEPTIDE [40-60] ADH AND ROLE OF PRO MUTANTS

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Rapidly increasing computational power has made molecular dynamics (MD) simulation a powerful tool for studying the structure and dynamics of biologically important molecules. The peptide residues 40–60 (YSGVCHTDLHAWHGDWPLPVK) of ADH, that has anti-aggregating properties against denaturing substrate proteins and known as intra-molecular chaperone peptide was simulated based on Gromacs MD program. Herein the pdb structure 2HCY (40-60) was used as initial conformation for molecular dynamics simulation. Native peptide simulated 10 ns with 1fs of time steps at 300K. The final geometry was used as initial geometry for mutations. The His (45, 49, 52) residues that were already reported as important residues for its chaperon action- were mutated to Pro independency, as Mute 1, Mute 2, and Mute 3 respectively. After 10 ns simulation of mutant peptides at the same condition the biophysical properties of them was analyzed and compared with the native one. Data showed that Pro residues disrupted 3D conformation throughout our simulation. Pro mutations, affect the hydrogen bonding network, gyration radius and hydrophobic surfaces that are major factors for chaperon action. While in native form, His residues allow the possibility of interacting with other residues to form especial secondary and 3D conformations that lead to the chaperon activity. This finding suggests that secondary and 3D structures are critical to the prevention of aggregation and having chaperon action. Then it was shown that the prolins do not play a major role in the chaperon action, whereas it is believed that Pro is the major effective residue in many chaperon proteins.

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STRUCTURAL STUDY OF IMMUNOGLOBULIN G SOLUTION AFTER PASTEURIZATION WITH AND WITHOUT STABILIZER

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Human blood and highly purified plasma derivatives such as immunoglobulin may contain viruses, which could be transmitted to recipients of transfusion. Nearly all viruses that exist in plasma-derived products can be destroyed by pasteurization which involves heating the products at 60º C for 10 hours. Purified human immunoglobulin G (IgG) in solution irreversibly aggregates to form an insoluble precipitate, when heated at high temperatures or for long periods of time. However protein aggregation can be prevented or reduced by changes in conditions or addition of co-solutes and/or additives. Pasteurization was investigated as a method of inactivating virus during the preparation of immunoglobulins for intravenous administration.The effect of pH, protein concentration and the presence of protein stabilizers on the structure of IgG molecules during pasteurization were investigated using an immunoglobulin solution derived from a Cohn's fraction II preparation. Changes in the secondary and tertiary structure of IgG molecules, as well as the degree of polymerization of the protein were investigated using spectrophotometery, circular dichroism and size exclusion chromatography. Only slight changes in secondary and tertiary structures were observed after pasteurization in a 10 g/L immunoglobulin solution at pH 4.5 and 5.5 in the absence of stabilizer and also in a 50 g/L immunoglobulin solution at pH 5.5 in the presence of glycine and sucrose/sorbitol. Concentrations of immunoglobulin solution below 20 g/L were not denatured when pasteurized at pH 4.5 in the absence of stabilizers. High concentrations of immunoglobulin solution required stabilizers such as glycine and sorbitol or sucrose to prevent or reduce denaturation during pasteurization.

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FUNCTIONAL AND QUANTITATIVE ANALYSIS OF HEAT SHOCK PROTEIN (HSP70) IN THE PRESENCE OF HIGH GLC CONCENTRATIONS, IN BOTH IN VIVO AND IN VITRO SYSTEMS

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Heat shock proteins (HSPs), also called stress proteins are a group of proteins that are present in all cells in all forms of life. They are present in cells under perfectly normal conditions and have the chaperone like activity, making sure that the cell's proteins are in the right shape and in the right place, hence, they are named "protein chaperones". HSP70 is one of the major stress protein families found in a variety of organisms, and these molecules play central roles in protein folding. Some studies have shown that expression of these chaperones is reduced during the course of diabetes, and such alterations may result in the onset of complications of diabetes. In the present study we determined serum concentrations of HSP70 in diabetic rats (STZ-induced diabetes mellitus) and in the normal group. The results showed a decrease in the serum levels of HSP70 due to diabetes induction as compared with the normal group. Our in vitro results indicated the nonenzymatic glycation of HSP70, thus its conformational changes which resulted in its inactivation. The functionality of native and glycated HSP70 was investigated in an enzymatic reaction using Leuciferase.

Nutrition

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ANTIOXIDANT EFFECTS OF IRANIAN RED GRAPE (RIBES RUBRUM) ON HOMOCYSTEINE, APOLIPOPROTEINS, HDL-C, AND LIPOPROTEIN(A) LEVELS IN HUMAN SERUM

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Red grape juice is a rich source of flavonoids such as catechin, epicatechin, quercetin, and anthocyanines with antioxidative effects. Flavonoids have a wide range of biological effects like anti-ischemic, anti-coagulative, and anti-lipoperoxidative effects on human body. Antioxidants that are strong enough to reverse oxidative reactions could scavenge free radicals and prevent lipid peroxidation processes. In the current study homocysteine, apolipoprotein A1 and B, as well as Lp (a) levels were measured in serum before and after consumption of Iranian red grape juice.The results showed that after consumption of red grape juice Lp(a) and homocysteine levels decreased significantly and the levels of HDL-C significantly increased ($P<0.05$).On the other hand, the levels of Apo A1 & Apo B did not change significantly ($P>0.05$). Decrease in homocysteine and Lp (a) levels and increase in HDL-C levels after consumption of red grape juice are evidence of its positive effects in decreasing factors leading to atherosclerosis and increasing factors that prevent atherosclerosis risk. Therefore, red grape juice consumption could decrease lipid peroxidation risk, as well as, the risk of initiation and progress of cardiovascular disorders.

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ANTIOXIDANT EFFECTS OF IRANIAN POMEGRANATE JUICE ON PARAOXONASE ENZYME ACTIVITY , SERUM HDL-C AND URINARY 8-HYDROXY 2-DEOXY GUANOSINE LEVELS

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Pomegranate juice is a rich source of antioxidants like tanins, anthocyanines, soluble polyphenols, and flavonoids. Pomegranate juice has anti sclerotic effects and can prevent cardiovascular disorders. Paraoxonase enzyme (PON) existing in pomegranate juice is a HDL-related esterase that both prevents lipid peroxidation process and hydrolyzes lipid peroxides in oxidized lipoproteins and atherosclerotic ulcers. Free radicals and other reactive groups are created naturally in vivo in physiologic and pathologic conditions, and most of them can cause oxidative damage of DNA that lead to DNA mutation and cancer. 8-hydroxy 2-deoxy guanosine (8-OHdG) is the most important product of DNA oxidation in genomic DNA of mammalian cells. In the current study, we investigated antioxidative effects of Iranian pomegranate juice on the activity of PON and levels of HDL-C and 8-OHdG. 3 drinking cups (750 cc) per day of pure pomegranate juice (prepared from Urmia Sarouneh Company) were consumed by 26 healthy individuals with an age range of 25-60 years for 1 month. The results for PON , HDL-C and 8-OHdG were significant($P<0.05$).The results indicate that the level of HDL-C and PON activity are increased and 8-OHdG level is decreased after consumption of Iranian pomegranate juice. Increase in paraoxonase after consumption of pomegranate juice can lead to a decrease in lipid peroxidation and an increase in HDL-C levels that has parallel effects on paraoxonase activity. Decrease in 8-OHdG reveals that oxidative stress and free radical levels as well as DNA damage is decreased after pomegranate juice consumption.

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THE EFFECT OF TART CHERRY JUICE CONSUMPTION ON SERUM APOA1, APOB AND HOMOCYSTEINE CONCENTRATIONS AND URINE 8-ISOPROSTANE LEVELS

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Cherries are reported to have naturally bioactive components. Cherries not only contain significant levels of antioxidants, but they provide a unique combination of antioxidants that are not found in other fruits. Phenol compounds in cherries may offer protection against heart diseases and stroke. The purpose of

this study was to determine the effect of consuming tart cherry juice on serum homocysteine, apoA1 and apoB concentrations and urine 8-isoprostane levels. 3 drinking cups (3×250 cc) per day of pure tart cherry juice were consumed by 27 healthy individuals with age range of 25-60 years for 28 days. Blood and urine samples were taken before and after consumption. Serum samples were examined for apoA1, apoB and homocysteine concentrations and urine samples were examined for 8-isoprostane levels. After examination, decrease in serum apoB, homocysteine and urine 8-isoprostane levels were significant ($P<0.05$), but increase in apoA1 concentration was insignificant ($P>0.05$). Supplementation of diets with tart cherry juice which contain flavonoids, will reduce serum apoB and homocysteine and urine 8-isoprostane levels. These diets provide health benefits and offer protection against cardiovascular diseases.

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STUDY OF THE ANTIOXIDANT ACTIVITY OF FOUR KINDS OF CULTIVATED RICE GRAINS IN MAZANDARAN PROVINCE (IRAN)

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Antioxidants in rice are important for human health. However, the level of antioxidants of different rice grains (Oriza sativa L.) which is the staple food in Mazandaran province of Iran and is the main agricultural product exported to other countries has not previously been reported. In this preliminary investigation, the in vitro antioxidant activity of the alcoholic extract of four different kinds of rice grains has been determined by ABTS/methemoglobin method and compared with Trolox, a vitamin E analog. It was found that the antioxidant activity (TEAC) in μmol per g of dry rice varied from the highest to the lowest as follows: Tarom rice (20.22), Khazar rice (9.44), Neda rice (8.78) and Sadri rice (1.33), respectively. TEAC was the highest in Tarom rice, followed by Khazar rice. This property may be due to the high contents of rice anthocyanins, vitamin E, tocotrienols and oryzanol. The functional chemistry, nutritional value and health benefits of antioxidants contained in rice grains, rice bran and their products should be intensively studied and characterized for their ingredients and stability.

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A COMPARATIVE STUDY OF SERUM AND ADIPOS TISSUE FATTY ACIDS IN PATIENTS WITH TYPE 2 DIABETES AND NORMAL SUBJECTS

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Diabetes is a heterogeneous disease which results from complex reactions of heredity, nutrition and lifestyle. Some studies have shown that a high intake of saturated fatty acids (SFA) increases the risk of type 2 diabetes, while polyunsaturated fatty acids (PUFA) decrease diabetes incidence by increasing insulin affinity to the receptors. In this study, serum fatty acids and adipose tissue composition as a long-term biomarker for fatty acid intake are determined, and their correlation with type 2 diabetes is investigated. The fatty acid composition of fasting serum and adipose tissue was studied in 98 patients with type 2 diabetes and 76 healthy control subjects using gas-liquid chromatography. The percentages of palmitic acid and positional isomer of oleic acid (11C-18:1) in adipose tissue of the patients were higher than the control group ($P=0.01$, and $P=0.02$, respectively). The percentages of palmitic acid, total saturated and monounsaturated fatty acids in the serum of the patients were also higher than the control group ($P=0.001$, $P=0.006$, and $P=0.001$, respectively). Mean concentration of triglycerides in the patients was higher than the control group ($t=6.7$, $P=0.001$). There was a negative correlation between serum PUFAs and cholesterol to HDL ratio and a positive correlation between serum SFA and TG in serum Large amounts of palmitic acid 11C-18:1 in adipose tissue may increase the risk of type 2 diabetes and it seems that patients with type 2 diabetes can have proper control over lipid parameters by having a higher intake of polyunsaturated fatty acids than saturated fatty acids.

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FATTY ACID CONTENT OF CANNABIS SATIVA L. SEED (HEMP SEED) CULTIVATED IN KHORASAN PROVINCE OF IRAN: FRIEND OR FOE

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Cannabis sativa L. is considered as a nutritional and a narcotic plant and has been a source of fiber, food, oil, medicine, and narcotics since prehistoric times. This study was conducted to evaluate fatty acid composition in the seed oil of Cannabis sativa L., which is cultivated in Khorasan province of Iran. Lipids were extracted with heptane in a straight through extractor. The triglycerides were transesterified to methyl esters with potassium hydroxide in methanol according to ISO method 5509. Fatty acid methyl ester composition was determined on two different gas chromatographs, Hewlett-Packard HP5890 and HP6890. The extracted seed oil contained significant amounts of oleic acid (52.86%), linoleic acid (25.17%), and palmitic acid (12.35%), which are the major fatty acids in this variety. On the other hand, γ- linolenic (1.14%), stearic acid (5.15%) and arachidic acid (1.83%) were low. The sum of all saturated fatty acids is 19.33% and the amount of unsaturated fatty acids is 79.17%. The high content of polyunsaturated fatty acids of hemp seed oil makes it beneficial to cardiovascular health but pregnant women and nursing mothers should avoid supplemental hemp seed oil. Because of possible antithrombotic activity of hemp seed oil, those with hemophilia and those taking warfarin should be cautious.

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EXPERRIMENTAL STUDY OF OXYTETRACYCLINE RESIDUE IN LIVER AND KIDNEY OF BROILER CHICKENS

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Background: Antibiotics are used for the remedy and prevention of diseases. Therefore the presence of antibiotic residues in liver and kidney of hens for human consumption should be considered seriously and this investigation was carried out to study antibiotic residues in the liver and kidneys of chicken after feeding antibiotics.Materials and Methods: Two hundred and sixteen Ross broiler chicken were divided into 4 groups with 3 three replicates. Oxytetracycline was added to the basal diet at 400, 800 and 1200 ppm levels. A control group receiving no antibiotic was also included. Experimental diets were fed to 30- 37 day old chicken. Animals from each cage were selected randomly during the last day of antibiotic feeding and ten days after feeding diets without the drug. Liver and kidney samples were collected for analysis. The presence of the antibiotic residues in liver and kidneys were measured by Delvotest kit Results: Liver and kidneys were examined and the result showed antibiotic presence in liver and kidneys from last day of feeding antibiotic to the 800 and 1200 ppm groups and over the next 7 days of feeding the diet without antibiotics.. Data were analysed by Chi square and Student t- test. No antibiotic was present in liver and kidneys of all other groups. Conclusion: It seems that chicken receiving antibiotics in their diets demonstrate the presence of this drug seven days after withdrawal.

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ANTIOXIDANT EFFECTS OF VITAMINS C AND E ON THE OXIDATIVE STRESS IN RAT HEPATOCYTES INDUCED BY T-BUTYL HYDROPEROXIDE

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Free radicals especially reactive oxygen metabolites can damage DNA, protein, enzymes, and membrane lipids. Lipid peroxidation in hepatocyte membrane may be involved in hepatic diseases. In this study, the effects of vitamins C and E on the oxidative stress in rat hepatocytes induced by t-butyl hydroperoxide was determined. First, rat hepatocytes were selected and incubated in the presence of t-butyl hydroperoxide and the amount of malondialdehyde, as a marker of lipid peroxidation, was determined. Then, this reaction was performed in the presence of various concentrations of vitamins C and E, and the malondialdehyde level was determined. The results of this study showed that the level of malondialdehyde in the presence of vitamins C and E decreased significantly ($P<0.05$), and vitamin E alone at 200μM concentration had the most inhibitory effect (56.8%). This study showed that vitamins C and E are useful in prevention of hepatic dysfunction.

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A COMPARATIVE STUDY OF PLASMA VITAMIN C (ASCORBIC ACID) CONCENTRATION OF NORMAL INDIVIDUALS AND SENILE CATARACT PATIENTS

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Aim and Methods: Senile cataract is an important public disease in the world. Every year, millions of cases of blindness worldwide result from unoperated cataract.The present study was performed to compare the plasma level of ascorbic acid in cataract patients and control subjects using dinitrophenylhydrazine spectrophotometric method. Results: Fifty cataract patients were compared with a control group (N=50) matched according to age. Statistical analysis of vitamin C concentrations in the plasma samples of the patients with cataract as well as those of healthy people revealed a decrease in plasma ascorbic acid levels in cataract patients ($P=0.0001$). Conclusion: This study showed that plasma vitamin C concentration in cataract patients is lower than the normal individuals. Vitamin C has a major role in prevention of age-related cataract.

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PALMITIC ACID UPREGULATES ICAM-1 AND VCAM-1 IN HUMAN BONE MARROW ENDOTHELIAL CELLS (HBMEC)

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Activated arterial endothelial cells (inflamed cells) overlying atherosclerotic lesion, express many of the same cell-surface moleculessuch as intercellular adhesion molecule-1(ICAM-1) and vascular cell adhesion molecule- 1 (VCAM-1).in post-capillary venules. These molecules have important roles in recruitment of leukocytes to endothelium and plaque formation. Saturated fatty acids are synthesized by both plants and animals from acetyl coenzyme-A as a form of long-term energy storage. Palmitic acid is a common 16-carbon saturated fatty acid that represents 10-20 % of the normal human dietary fat intake. Palmitic acid is mostly found in cookies, nuts, crackers, french fries and microwave popcorn. Because this fatty acid exists in high levels in Iranian diets, we investigated whether this fatty acid can induce expression of ICAM-1 and VCAM-1 in human bone marrow endothelial cells (HBMEC). HBMEC incubated whit tumor necrosis factor-α (TNF-α) and lipopolysaccharide (LPS) for an additional 18-h alone, then in the presence of palmitic acid for 24-h before assessing the expression of the VCAM-1 and ICAM-1. The expression of adhesion molecules in HBMEC was assayed by ELISA and western blotting. After 18-h incubation of the cells with 10μg/ml LPS or 12-h incubation with 0.001μg/ml TNF-α and further 24-h incubation with palmitic acid, VCAM-1 and

ICAM-1 expression was increased in both soluble and membrane forms. The results suggests that higher intake of diets enriched in palmitic acid can increase expression of ICAM-1 and VCAM-1 in endothelial cells and sustain recruitment of leukocytes in the site of inflammation.

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COMPARISON OF SERUM TOTAL ANTIOXIDANT LEVELS AND DIETARY INTAKE OF ANTIOXIDANTS BETWEEN MULTIPLE SCLEROSIS PATIENTS AND HEALTHY SUBJECTS

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Objective- Multiple Sclerosis (MS) is a chronic, immune-mediated inflammatory and neurodegenerative disease of the central nervous system (CNS), with an etiology that is not yet fully understood. Increasing evidence shows that oxidative stress plays an important role in the pathogenesis of MS. The aim of this study was to compare dietary intake of antioxidants and serum levels of total antioxidant status (TAS) in MS patients with that of normal subjects. Methods: serum levels of TAS and dietary intake of the main antioxidants of 21 MS patients (16women) were compared with age and gender matched healthy controls. Serum samples were collected and frozen for further spectrophotometric analysis. Food frequency questionnaires and 24-hour dietary recall for 3 days of all subjects were obtained. Results: There was no significant difference in serum levels of TAS between two groups. No significant difference was seen in consumption of dietary antioxidant between them. Additionally, daily intake of vitamin C, vitamin E, vitamin A, folate and dietary fiber were not significantly different between groups. Conclusions: Evidence bearing on the possible relation and benefits of dietary antioxidant in MS is lacking. More research is required to assess the effectiveness of diet interventions on antioxidant status in MS.

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RESPONSE OF BIOCHEMICAL MARKERS OF BONE TURNOVER TO SOY ISOFLAVONES CONSUMPTION IN POSTMENOPAUSAL WOMEN

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Introduction: Ovarian hormone deficiency is a major risk factor for osteoporosis in postmenopausal women. Phytoestrogens are plant products with weak estrogen-like activity and some evidence suggests that these compounds may have possible inhibitory effects on bone loss. Phytoestrogens come in several forms, a major subclass being the isoflavones. Isoflavones, in particular daidzein and genistein are highly enriched in soy compared with other food sources, and therefore most soy foods will provide a significant dietary source of these bioactive nonnutrients. Materials and Methods: This study was a "before and after treatment" clinical trial on 15 postmenopausal women 45-64 years of age with osteopenia, between 1 to 10 years of menopause duration and without gastrointestinal, renal, cardiovascular and thyroid disease. The subjects were asked to consume 35 gram/day of soy protein containing approximately 98.3 mg/d isoflavones (sum of all kinds of isoflavones) for 12 weeks. Blood and urine sampling, anthropometric measurements and a 2-day dietary recall were done at the beginning of the study, and after 6 and 12 weeks. Results: Soy isoflavones consumption showed a significant reduction (27 percent) in deoxypyridinoline and a significant rise (18 percent) in total alkaline phosphatase. There were no significant differences in serum osteocalcin, c-telopeptide, insuline-like growth factor binding protein 3 and type-I-collagen telopeptides. Conclusion: Preventive measures are the best and the most cost effective means to minimize osteoporosis. Considering the effects of soy protein consumption on bone turnover especially bone resorption, dietary intake of this food item can suppress bone loss in postmenopausal women and could be recommended for the prevention of osteoporosis.

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COMPARISON OF DIETARY PATTERNS BETWEEN PATIENTS WITH MAJOR DEPRESSIVE DISORDER AND HEALTHY SUBJECTS

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Introduction: There has been a limited finding on food preference and dietary pattern among patients suffering from major depressive disorder (MDD). Objective: This study was carried out to compare the food pattern of major depressed patients with healthy subjects.Methods: This study was a cross-sectional research carried out on 63 patients during autumn 2006 in Milad clinic, Ahwaz. Patients were 15-50 years old with major depression (36 female) and their dietary patterns were compared with that of 65 healthy age and sex matched individuals as control group. Questionnaires consisted of 13 questions of Beck test, food frequency questionnaire (FFQ) and information about demographic characteristics. Body Mass Index was also calculated.Results: Female patients had higher mean BMI than their counterpart controls (22.8±4.3 vs 21.1±2.5; $p<0.05$). Consumption of milk and dairy products, fresh vegetables, nuts and olive were 1.5, 1.8, 2 and 4 times more in controls than patients, respectively ($p<0.05$). Weekly intake of legumes ($p<0.01$) and vegetable oils ($p<0.01$) were more prevalent in the control group than patients with major depression. It was also observed that patients used to eat a lower amount of sea foods than controls (57.4% vs 81.5; $p<0.01$). Moreover, patients used to limit their meals to less than 3 times a day, compared to the controls ($p<0.001$). However, patients used to eat more sugar ($p<0.05$). Conclusion: It was seen that patients consume lower amounts of sea foods than controls. On the other hand, patients consume higher amounts of sugar than healthy matched people. In general, depressed patients, and in particular,

female patients have poor nutritional pattern. Therefore, correction of food pattern is of importance in such patients.

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CORRELATION OF HUMAN SENILE CATARACT WITH SOME BLOOD CONSTITUENTS

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purpose: The causes of age-related cataract are multifactorial and particular consideration has been given to the role of nutritional factors in cataract formation. The purpose of this study was to determine the levels of certain blood constituents in cataract patients and controls and evaluate their potential, if any, as identifying risk factors in cataractogenesis.methods: This investigation is a pair matched case-control study where 155 cataract patients and 155 matched controls were chosen for evaluation from March 2000 to March 2001 in Hazrate Rasoul Akram hospital in Tehran. .Age of cases and controls were up to 40 years. Demographic and clinical characteristics of all patients and controls were collected in a questionarre. Cataract subjects were further divided into subgroups depending on the location of the cataract. All underwent routine eye examinations and blood samples were taken from each one for blood biochemistry analysis including serum levels of glucose, urea, cholesterol, triglycerides, total protein, albumin, bilirubin, calcium, sodium and potassium and the enzymes aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase. Na^+ and K^+ were measured by flame photometry and the other analytes were measured by colorimertic methods. All information and data were analysed by version 10 SPSS program.RESULTS: Mean levels of total protein, bilirubin, calcium, sodium, alanine aminotransferase and alkaline phosphatase differed significantly between patient and control groups. Patients with posterior subcapsular cataract had significantly higher glucose levels ($p = 0.05$) compared with patients with other types of cataract. It was also found that those patients with a cortical cataract had the highest level of urea, differing significantly from other subgroups and controls. conclusions: The mean levels of all constituents under study fell within normal body range. However, the levels of several important factors demonstrated a positive and significant correlation with cataract. While we do not presume to present these results as conclusive, it is possible that a gradual and constant variation in these parameters may be predisposing factors in cataractogenesis.

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STUDY OF THE CALCIUM BIOAVAILABILITY OF CALCIUM ACETATE AS COMPARED WITH CALCIUM CARBONATE

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Introduction: Osteoporosis is a life threatening disease. One of the important etiologies of osteoporosis is low calcium intake from daily diets during adulthood and growing age. So calcium salt administration in pharmacological form or as a food additive, is recommended as a protective mechanism for this disease especially in women. Calcium acetate is a water soluble agent whereas calcium carbonate (which is routinely used in pharmacological forms of Calcium) is not easily dissolved in water, so we decided to compare the bioavailability of calcium carbonate with calcium acetate. Materials and Methods: During a clinical trial study, 20 normal female volunteers entered the study according to the inclusion criteria. In the day of study, fasting urine specimens were collected from all subjects. 2 hours after a meal, a solution of calcium carbonate was administered to all participants and then urine specimens were collected for 4 hours. After a 10 day washout period, this test was repeated with calcium acetate. The results were analyzed with paired t test. Results: mean increase in urine calcium was 38.55±12.2 mg/dl and 36.33±12.28 mg/dl after administration of calcium carbonate and calcium acetate, respectively.There was no statistically significant difference between calcium absorption from calcium carbonate and calcium acetate. CONCLUSION:: Regarding the beneficial effects of calcium acetate as a soluble salt , in comparison with calcium carbonate and according to the results of this study indicating the equivalency of calcium absorption from these two salts, calcium acetate can be administered as an appropriate substance for prevention and treatment of osteoporosis. Indeed it can be used in enriching the liquid comestibles.

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DEFICIENCY OF FOLIC ACID, VITAMINS B6 AND B12, HYPERHOMOCYSTEINEMIA, METHYLENE-TETRAHYDROFOLATE REDUCTASE 677 C>T DIMORPHISM AND CORONARY ARTERY DISEASE IN PAKISTAN

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Pakistani people have the highest known rate of coronary artery disease (CAD). The possibility of a correlation between vitamin deficiency and hyperhomocysteinemia in Pakistanis with acute myocardial infarction (AMI) was examined in two case control studies involving 408 AMI patients and 289 normal healthy subjects from Karachi and Rawalpindi. Fasting venous blood was obtained from cases and controls. Serum/plasma was analyzed for folic acid, pyridoxal phosphate (PLP, coenzymic form of B6), B12 and homocysteine. DNA was isolated from leukocytes for a study of methylenetetrahydrofolate reductase (MTHFR) dimorphism 677 C>T. MTHFR alleles were determined by assays based on polymerase chain reaction and restriction endonuclease analysis. Nutrient values for all subjects were based on current WHO recommendations. Compared to controls, there were significant deficiencies of folate (30.3% vs 64.9%), B12 (7.3% vs 63%) and PLP (44.6% vs 73.9%) in AMI patients in Karachi-based population. In Rawalpindi-based population, only B12 was significantly deficient in AMI patients compared to controls (38.2% vs 4.5%). Mean plasma homocysteine levels in AMI patients in both populations

(17.9±8.2 µmol/l and 19.8±8.8 µmol/l) were insignificantly higher than mean levels in controls (16.9±6.1 µmol/l and 19.4±10.6 µmol/l). These mean homocysteine concentrations are among the highest reported in the scientific literature. The MTHFR 677C>T dimorphism was not associated with MI (x2=0.25, 1 df, P=0.82). Substantial nutrition deficiencies of folate, B6 and B12, along with mild hyperhomocysteinemia, appear to be increasing the risk of CAD in Pakistani population. Higher incidence of AMI in Pakistan occurs through mechanisms other than MTHFR related pathways.

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EFFECT OF SHORT-TERM TREATMENT OF ESSENTIAL OIL OF SALVIA HYPOLEUCA L. LEAVES ON SERUM GLUCOSE AND INSULIN LEVELS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Recently, there has been increasing interest in the use of medicinal plants. The plant kingdom has become a target for the search by multinational drug and biologically active lead compounds. Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes. The hypoglycaemic activity of a large number of these plants has been evaluated and confirmed in different animal models. The leaves of Salvia hypoleuca L. (Lamiaceae) are reported to have a wide range of biological activities, such as tonic, carminative, digestive, antispasmodic and anti-inflammatory effects in traditional medicine. To determine the hypoglycaemic effect of the plant, we investigated the effects of essential oil of the plant on healthy and streptozotocin-induced diabetic rats. The animals were made diabetic using streptozotocin (70 mg/kg, i.p.). The essential oil (0.2, 0.4 and 0.5 ml/kg) were injected intraperitoneally. The control groups were administered sunflower oil as vehicles of essential oil. Blood samples were obtained from retro-orbital sinus before administration and 1, 3 and 5 h after administrations. The serum glucose was measured by the enzymatic method of glucose oxidase. The results showed that the essential oil of sage decreased serum glucose, while increased insulin levels. The present data indicate that sage essential oil has hypoglycaemic effect on diabetic animals and the plant should be considered in future therapeutic researches.

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EFFECT OF TOMATO JUICE ON BLOOD GLUCOSE LEVEL AFTER ALLOXAN INJECTION IN RAT

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Tomato is widely consumed as a vegetable and as processed tomato products (juice, sauce, soup and ketchup). The active compounds isolated from tomatoes that have antioxidant properties include lycopene, β-carotene, vitamin C and flavonoids. The present study was to examine the influence of oral administration of tomato juice on the level of serum glucose after alloxan injection. Female and male Wistar rats were randomly allocated to three groups of 16 rats (8 female and 8 male) each. The first group assigned as control and received tap water (8 ml/kg/day). Two remaining groups received fresh tomato juice or preheated tomato juice (8 ml/kg/day), for 14 successive days via feeding tube. Hyperglycemia was induced by the S.C. injection of alloxan (100 mg/kg B.W.). Feeding was continued for another 3 days. Fasting blood glucose concentration in serum sample was measured before and after treatment. Statistical significance was determined using t-test or ANOVA, a value of P less than 0.05 was statistically considered significant. No statistically significant difference was found in serum glucose between groups before and after treatment (P more than 0.05). In addition, no statistically significant difference was found in the serum glucose level between the females and males in each group (P more than 0.05). The percentage of change in blood glucose was not affected by tomato juice treatment (P more than 0.05). Our findings showed that absolute and percentage of change in fasting blood glucose was not affected by tomato juice treatment after alloxan injection in rat.

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EFFECTS OF CALCIUM SUPPLEMENTATION ON SERUM LIPOPROTEINS IN OBESSE ADULTS RECEIVING ENERGY RESTRICTED DIET

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Background: Many of studies suggested that calcium intake might affect serum lipid concentration but these studies had short term, were uncontrolled and the others showed no effect. Objective: Our objective was to determine the effect of long term supplementation of calcium carbonate on circulating lipids in calorie restricted obese healthy adults.Subjects and Methods: We performed a double blind randomized placebo - controlled trial in 40 adults with BMI > 25 kg / m2 who were 20 - 60 years of age . Subjects who were maintained on balanced deficit diet (- 500 kcal / d) , randomly assigned to two groups with 1000 mg Ca / d as calcium carbonate , or placebo for 24 weeks . Fasting lipoprotein concentrations including total cholesterol, HDL - C, and triacylglycerol were obtained at baseline, and after 12th and 24th weeks. TC, HDL-C, TG were measured by enzymatic methods. LDL- C, and VLDL - C were estimated by Friedwald formula. Results: There was no significant difference in variables at 12 and 24th weeks between two groups. But TC, LDL-C decreased significantly at 12 and 24th weeks in two groups at the end of study compared to initial values ($p < 0.05$). Similarly TG and VLDL -C were significantly decreased in two groups compared to initial values, but only in calcium group there wasa significant difference at 24 th weeks compared to beginning ($p < 0.05$).Conclusion: Data from this study suggest that 24 weeks of supplementation with 1000 mg Ca /d did not have any effect on serum lipoproteins beyond what can be achieved in a energy restricted diet.

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DETERMINATION OF HUMAN MILK CALCIUM, PHOSPHORUS AND MAGNESIUM AND EFFECTIVE FACTORS ON HUMAN MILK IN WOMEN REFERRING TO HEALTH CARE CENTERS OF NORTH AND SOUTH OF TEHRAN

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Aside from being a complex mixture, mother's milk is designed and carefully tailored according to the needs of infants. Studies have shown that mother's nutrition can affect biochemistry of her milk as in malnourished mothers, protein proportions decline to two thirds of the normal and increases if protein is added to their diet. Mothers' nutrition affects both their milk nutrients and the growth and development of the newborns. All mothers referring to selected health centers of north and south of Tehran were selected to participate in the study. Calcium, phosphorus and magnesium levels in mothers' diet were determined using a 24 h diet history questionnaire for 3 days. Ziest Chemie diagnostic kits were used to measure calcium, phosphorus and magnesium contents of milk samples. Assessment and comparison of biochemical components of mother's milk and the possible variables (including, age, height, weight, occupation, education, economical situation, physical activity, duration of breast feeding, and diet) between the two health centers of north and south of Tehran show that the majority of mothers in both north (44.4%) and south (46.65%) had 20-25 years of age. The average mother's age in northern Tehran was (25.75) and in southern Tehran was (25.24). No significant difference was observed (P=0.231). The weight of mothers within two groups of north (31.5%) and south (34.6%) was within the range of 55-64 Kg, The average mothers' weight in northern Tehran was 67.67 and in southern Tehran was 64.82 and a significant difference was observed (P=0.004). The average mothers' height in northern Tehran was 158.68 and in southern Tehran was 158.19 and no significant difference was observed (P=0.53). The average level of calcium in mothers' milk in north and south of Tehran was 13.36±0.18 and 11.5±0.11 mg/dl, respectively. The average level of phosphorus in mothers' milk in north and south of Tehran was 5.7±0.14 and 4.7±0.08 mg/dl and the average level of magnesium in mothers' milk in north and south of Tehran was 2.16±0.05 and 2.25±0.13 mg/dl, respectively and the average level of calcium in mothers' diet in northern and southern groups were 770.5±26.5 and 793.6±25.5 mg/day, respectively and the average level of phosphorus in mothers' diet in northern and southern groups were 790.3±21.8 and 915.2 mg/day. The average level of magnesium in mothers diet in north and south of Tehran was 135.8±4.5 and 167.1±4.2 mg/day. Compared to other populations, the levels of calcium, phosphorus and magnesium were lower. There was no significant relationship between the levels of calcium, phosphorus and magnesium of milk with those of diet (P>0.05).

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FATTY ACID COMPOSITION OF IRANIAN WALNUT CULTIVARS

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Regular consumption of walnuts (Juglans regia) can have beneficial effects on coronary heart disease (CHD). In the prsent study, the fatty acid (FA) composition of five Iranian walnut cultivars were investigated and compared. Walnut oils extracted by Soxhlet and the FAs were estrified by sodium methoxide.The corresponding fatty acid methyl esters (FAMEs) were identified and measured by gas chromatography (GC). The predominant FA found in walnuts (all cultivars) was linoleic acid,in accordance with the findings of other similar investigations. Poly unsaturated fatty acids (PUFAs) were the major group of fatty acids, ranging from 58.9%-63.33%. FA quantity differences between walnut cultivars were statistically significant ($p < 0.05$) even though (omega-6/omega-3)ω6/ω3 ratios were close between cultivars. Results obtained in the present study show the differences in FA composition among the different cultivars of Iranian walnut. The ω6/ω3 ratios were found to be close to the natural ratio, and therefore, consumtion of Iranian walnuts may be helpful to CHD patients.

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THE EFFECT OF FRESH GARLIC ON SERUM LIPID, BLOOD SUGAR AND SOME HORMONES THAT INVOLVE IN SUGAR AND LIPID METABOLISM IN HYPERGLYCEMIC AND/OR HYPERLIPIDEMIC INDIVIDUALS

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Background and Objective: Garlic has been known as a sugar and cholesterol lowering agent. In the present study the effect of fresh garlic on fasting blood sugar (FBS), lipids, thyroid hormones and serum insulin has been investigated in hyperglycemic and/or hyperlipidemic individuals.Materials and Methods: the volunteers (85) were divided into 3 groups as follows: group 1:30 individuals with FBS more than 126mg/100ml, group 2: 30 persons with serum cholesterol higher than 245mg/100ml, and group 3: 25 individuals with FBS more than 126mg/100ml and cholesterol higher than 245mg/100ml. Blood samples were collected before participation in the study and then the participants consumed raw fresh garlic (5g twice a day) for 42 days; second blood samples were collected, after that the individuals stopped any garlic consumption for next 42 days and the third blood samples were collected. Blood sera were separated and FBS, glycated hemoglobin (HbA1C), total cholesterol, HDL-C, LDL-C, triglyceridesTG), thyroid hormones (T3, T4 and TSH), and insulin were measured.Results: There were no

significant differences in group 1 after 42 days garlic consumption. In group 2, Cholesterol ($p<0.001$) and FBS ($p<0.01$) were decreased and HDL-C ($p<0.001$) was increased significantly after 42 days garlic consumption whereas Cholesterol ($p<0.001$), FBS and TG ($p<0.05$) were raised and HDL-C ($p<0.01$) was decreased significantly after 42 days of stopping garlic consumption but other blood biochemical factors did not change significantly. In group 3, there were reductions in FBS ($p<0.01$), HbA1C ($p<0.05$), and total cholesterol ($p<0.001$), but HDL-C increased ($p<0.05$) significantly after 42 days of garlic consumption. Conclusion: according to the result of present study, fresh raw garlic consumption can decrease FBS, cholesterol, HbA1C, and increase HDL-C, but has no effect on the serum levels of hormones that affect fat and sugar metabolism including thyroid hormones and insulin.

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STUDY OF THE EFFECTS VITAMIN E, LYCOPENE, QUERCETIN AND NARINGIN ON THE LOW DENSITY LIPOPROTEIN OXIDATION

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Because much data have accrued to support the concept that oxidatively modified low density lipoprotein (Ox-LDL) can promote atherogenesis, the role of antioxidants in decreasing LDL oxidation has assumed great importance. In this research, the effects of compounds including: vitamin E, lycopene, quercetin and naringin on the LDL oxidation were studied in vitro. LDL was isolated from normo-lipidemic human plasma by sequential ultracentrifugation. The rate of LDL oxidation by copper ions were determined in the presence and absence of each one of the above four compounds using two methods including conjugated dienes and thiobarbituric acid. The effects of agents used on the LDL oxidation showed that all had the inhibitory effect, and their potencies were: lycopene>naringin>vitamin E>quercetin. These compounds may have favorable effects in ameliorating atherosclerosis.

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TRYPTIC AND CHYMOTRYPTIC HYDROLYSIS OF CAMEL AND BOVINE WHOLE CASEIN FRACTIONS; A COMPARATIVE STUDY

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Bioactive peptides encrypted in milk proteins are of particular interest in food industry because of their many physiological roles. These peptide elements can be released or activated in vivo during gastrointestinal digestion or in vitro throughout food processing using specific enzymes. Therefore these peptides are a major source for the manufacture of novel functional food ingredients. This work was carried out for the purpose of measuring the digestibility of camel and bovine casein fractions using two digestive proteases namely trypsin and chymotrypsin. The hydrolysis experiments were performed in phosphate buffer and 37°C up to 3 hours. Protein and peptide profile were studied by sodium dodecyl sulphate polyacrylamide gel electrophoresis.To quantify degree of hydrolysis, a rapid, sensitive and convenient spectrophotometeric assay, using orthophthaldialdehydeOPA) as a reagent was performed. The results showed that camel whole casein was more susceptible to chymotryptic hydrolysis whereas bovine casein was more susceptible to tryptic hydrolysis. In the presence of both digestive proteases, camel casein fraction demonstrated a higher degree of hydrolysis. Caseins have an appropriate amino acid composition that is important for growth and development of nursing infants. It is well accepted that the nutritional value of proteins may differ substantially depending on their (essential) amino acid composition and digestibility. Since camel caseins as observed in this study revealed a higher digestibility in the presence of both digestive enzymes, it can be concluded that camel caseins is possibly a preferable alternative to the bovine caseins for formulation in the milk protein based infant formulae.

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EFFECT OF FOLIC ACID SUPPLEMENTATION ON PLASMA HOMOCYSTEINE CONCENTRATION IN PATIENTS WITH MAJOR DEPRESSIVE DISORDER

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A consistent finding in the major depressive disorder (MDD) has been a low plasma and red blood cell folate which has also been linked to poor response to antidepressants therapy. Elevated plasma homocysteine concentrations are recognized as a risk factor for MDD and inversely related to plasma folate levels.The present investigation was designed to investigate whether the coadministration of folic acid with antidepressant therapy would decrease the plasma homocysteine levels and enhance the antidepressant action of fluoxetine. 70 patients were randomly assigned to receive 20 mg fluoxetine together with either 1 mg folic acid or an identical looking placebo daily for 8 weeks.All patients met the DSM-IV-IR criteria for major depression and had a baseline Hamilton Rating Scale Score of 20 or more. Baseline and 8-week estimations of plasma folate and homocysteine were carried out. Patients receiving folate showed a significant increase in plasma folate.This was less in men than in women ($P<0.05$).Plasma homocysteine was significantly decreased in women by 32%, but there was no significant change in men. 88.9 percent of women, who received the folic acid supplement, showed a

good response (.50% reduction in score) as compared to 59.3% of women in placebo group (P<0. 05). These findings suggest that daily administration of 1mg folic acid effectively reduces plasma homocysteine concentration in women with MDD. Folic acid supplementation is a simple method for improving the antidepressant action of fluoxetine. Men require a higher dose of folic acid to decrease homocysteine levels than women , but more studies is required to ascertain the optimum dose of folic acid for decreasing homocysteine levels in blood.

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DETERMINATION OF THE GLYCEMIC INDEX OF MACARONI IN HEALTHY PERSONS

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The glycemic index (GI) is a measure of the food power to raise blood glucose (B-glucose) concentration after a meal. Recent studies indicate that the risks of diseases such as type 2 diabetes and coronary heart disease are strongly related to the GI of the overall diet. The aims of this study were determine the GI of macaroni (Salam Macaron, Kashan, Iran) and compare the GI of tested food in men and women. To determine the GI, measured portions of food containing 50 g of carbohydrates were eaten by 15 healthy volunteers (8 men and 7 women) after an overnight fast. B-glucose curves were constructed from B-glucose values at time 0, 30, 60, 90, 120 min after the meal. The GI was calculated by dividing the incremental area under the curve for the tested food by that for the standard food (same amount of glucose) and multiplying by 100. In each volunteer each food was tested 3 times so that 3 GI's was obtained and the average was calculated. The GI for tested food was calculated as the mean from the respective average GI's of the 15 volunteers. MS Excel, Graph Pad Prism 5 and the statistical program SPSS v. 13 were used to analyze the data. The mean value of the GI for macaroni was 50.18 ± 14.35 in the whole group of 15 volunteers. No significant difference could be seen between the GI in men and women. Our results indicate that macaroni has low GI, suggesting that macaroni may be an appropriate part of diets intended to improve control of blood glucose levels. An accurate standard method for the determination of GI needs to be defined, carefully used and re-evaluated to enable a comparison of the results with various methods of other working groups.

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IMPROVEMENT OF DIABETIC COMPLICATIONS WITH VITAMINS C AND B6

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Type2 diabetes is usually diagnosed after the age of 40. Type2 diabetes is frequently associated with insulin resistance and normal or even elevated insulin; although subnormal insulin levels are also observed in some type2 diabetics. Glucose regulation depends on a wide range of vitamins, minerals and other micronutrients. Supplementation with appropriate vitamins may therefore be of value in the prevention and treatment of diabetes. Recent studies showed that oral vitamins B6 and C supplementation in diabetic patients, improved glucose tolerance test. In the present study HbA1c, FBS and body weight were investigated in 75 patients over 40 years of age with type2 diabetes. These parameters were measured by standard and conventional methods before and after prescription of vitamins C (two 250 mg tablets daily) and B6 (two 40 mg tablets daily). With respect to all three parameters significant desirable changes took place 6 months after the onset of trial: Weight loss (80 kg versus 76) , HbA1c decrease (10.3 % versus 6.1) and FBS decrease (192 versus 107) were observed six months after supplementation with these vitamins(P<0.05). Our study suggests that vitamins B6 and C improve diabetic complications.

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SATURATED AND UNSATURATED FATTY ACIDS DISSIMILARLY REGULATE GENE TRANSCRIPTION

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Coronary heart disease (CHD) is the leading cause of death in developed countries and the link between dietary fat and heart disease has been clearly established, with saturated fatty acids being considered as atherogenic fat, whereas polyunsaturated fatty acids are cardioprotective. Many of the potentially beneficial effects of dietary fatty acids in preventing or inhibiting the progression of CHD are mediated through the control of gene transcription. In this study, the P19CL6 cardiac cell‐line was targeted for the investigation of (i) the effects of long chain fatty acids (LCFA) and clofibrate on mRNA levels of specific lipid metabolism related genes, such as heart type fatty acid‐binding protein (H‐FABP) and peroxisome proliferator‐activated receptors (PPAR a, b, g) in the P19CL6 cell ‐line, and (ii) to determine the effects of LCFAs and clofibrate on global transcriptome levels, using cDNA microarray analysis. Methods: After culturing P19CL6 cells with LCFAs or clofibrate, the total‐RNA was extracted and expression levels of H-FABP, PPAR a, PPAR b, and PPAR g genes were determined by RT‐PCR. In addition, microarray analysis was used to compare global transcriptome profiles in P19CL6 cells cultured with different LCFAs or clofibrate. Results: LCFAs significantly increased the abundance of PPAR a and PPAR g. Moreover, microarray analysis showed the effects of linoleic and a ‐linolenic acids and clofibrate were similar but differed from those of palmitic and oleic acids. Conclusion: These findings show cellular responses to polyunsaturated fatty acids differ from those observed with saturated and monounsaturated fatty acid.

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THE EFFECT OF SUPRAPHYSIOLOGICAL CONCENTRATIONS OF RUTIN, QUERCETIN AND

KAEMPFEROL ON TOTAL ANTIOXIDANT ACTIVITY OF HUMAN PLASMA

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Rutin(R), Quercetin (Q) and Kaempferol (K) are polyphenlic compounds whose main dietary sources are fruit and vegetables. Consumption of these compounds has been shown to protect against heart disease and cancer and it could be linked to their antioxidant activity. To test the total antioxidant activity, (TEAC) assay was used in fresh human plasma by adding the different concentrations (10, 20, 50, 100 µmol/l of Rutin, Qurecetin and 100 µmol/l of kaemperol. Linear correlation and ANOVA were used as statistical methods. TEAC of fresh plasma without flavonoids was 1.39+0.04 mmol/l (n=10). Significant increases were observed when 50 or 100 µmol/l quercetin and 100 µM rutin and keamPferol were added to plasma ($p<0.001$). Linear correlation suggested a dose-related effect from 10 to 100 µmol/l for quercetin ($R2=98.4\%$, $P< 0.001$) and for rutin ($R2=95.3\%$, $P=0.005$).Using the TEAC assay to test antioxidants in fresh human plasma in supra-physiological concentrations, produced results, which could support a physiological role for flavonoids in the body's antioxidant defense system.

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NEW DESIGN FOR DETECTION OF ANTIOXIDANT ACTIVITY OF FLAVONOIDS USING HPLC AND ELECTROPHORESIS ON HUMAN LYMPHOCYTES, PLASMA, URINE AND DIET

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Flavonoids are polyphenolic compounds whose main dietary sources are fruit and vegetables. Consumption of flavonoids has been linked to protection against heart disease and cancers. To examine antioxidant properties of flavonoids using an ex vivo and in vivo tests on human tissue lymphocytes, comet assay was used in different concentrations (7.6 - 279.4 µmol/l), to examine oxygen-radical caused DNA damage in human lymphocytes. The antioxidant activity of flavonoids at the concentration of 279 µmol/l, was measured and ranked in decreasing order of potency as follows: luteolin -myricetin - quercetin - kaempferol - quercitrin (quercetin-3-L-rhamnoside) - apigenin - quercetin-3-glucoside - rutin (quercetin-3 β D-rhamnoside)-and vitamin C. Ranking was similar using estimated ED50. Also, ten stable type 2 diabetic patients were treated for 2 weeks on a low (76.3 mg) or high (110.4 mg) flavonoid diet. Oxidative DNA damage on the low and high flavonoid diets was 220+ 12 and 192 + 14 AU, respectively ($p <0.05$). By fasting plasma concentration ($r2 = 0.51$) or urinary excretion ($r2 = 0.75$) consumption of dietary flavonols could be predicted. It was shown that, at the concentrations of 300 µmol/l of flavonoids , total antioxidant capacity of major dietary flavonoids was greater than vitamin C. There was a strong positive correlation between the antioxidant activities of flavonoids with the numbers of hydroxyl group ($p<0.001$).

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EFFECT OF CITRUS LIMON BURM F. ON BLOOD PRESSURE IN HYPERCHOLESTEROLEMIC RABBITS

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Background: Hypertension has been known as a main risk factor for cardiovascular diseases (CVDs). Effect of antioxidants in CVDs prevention has been observed. Results of studies have shown their lipid lowering and also antihypertensive effects. Flavonoides are effective antioxidants in some vegetables and fruits. One of the fruits in our country which is consumed by all groups of people is Lemon (Citrus limon Burm f.). So this study aimed at finding the antihypertensive effect of this fruit in hypercholesterolemic rabbits.Methods and Results: 30 New Zealand White (NZW) rabbits were divided in three equal groups, control and intervention (2 and 3), randomly. Weight and serum lipids of all subjects were measured at the beginning of study. Then, all rabbits consumed an atherogenic diet (1% cholesterol plus regular diet) for 2 months. And for intervention (2) group we added 5 ml of fresh Citrus juice per day, and for intervention (3) group 1g of dried Citrus peal powder per day during the period of the study. At the end of the study we checked weight and serum lipids and systolic (SBP) and diastolic (DBP) blood pressures. Results of this study showed that mean DBP in three groups were as follows: group (2): 57.0±10.7, group (3): 55.3±19.2, and control group: 60.3±9.9 ($P>0.05$). Also mean SBP for our groups are 82.4±15.3, 76.0±25.1 and 82.1±11.7, respectively ($P>0.05$). Conclusions: These results establish that Citrus limon Burm f. don't have any effect on blood pressure in hypercholesterolemic rabbit.

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COMPARISON OF THE HYPOLIPIDEMIC EFFECTS OF THE AQUEOUS EXTRACT OF GARLIC AND SHALLOT ON FRUCTOSE-INDUCED INSULIN RESISTANT RATS

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The present study evaluated the effect of garlic and shallot aqueous extracts on glucose tolerance and lipid profiles in a

fructose-induced insulin-resistance rat model. Male albino Wistar rats were fed either normal or high-fructose diet for a period of eight weeks. Fasting blood glucose, triglycerides, cholesterol, LDL, FIRI, and the area under the intraperitoneal glucose tolerance curve (I.P. GTT) were significantly elevated in fructose-fed animals. Fructose-induced insulin resistant rats were treated with aqueous shallot or garlic extract (500mg/Kg BW/day, I.P.) for duration of twelve weeks. Control and fructose-fed animals only received normal saline (0.9%). The results showed that garlic and shallot extracts could not significantly improve the i.p. GTT and lipid profile at the fourth week after treatment. The intraperitoneal glucose tolerance has been significantly improved in fructose-induced insulin resistant animals after twelve weeks administration of garlic and/or shallot extracts. These results indicate that shallot and garlic extracts have a hypoglycemic influence on the fructose-induced insulin resistant animals. Garlic extract treatment, but not shallot extract, for a period of twelve weeks could significantly diminish the level of cholesterol and low density lipoproteins (LDL) in fructose-fed rats. The data suggest that intake of the garlic ameliorated the fructose-induced hypercholesterolemia in the insulin resistance state.

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EVALUATION ROLE OF MANNOSE - BINDING LECTIN GENE AND PROMOTER POLYMORPHISM IN SUSCEPTIBILITY TO INFECTIONS

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Introduction: Mannose-binding lectin is a member of the collection family which binds to various oligosaccharides and activates the classical pathway of complement independent from C1q; the aim of present study was to determine the distribution of the alleles of mannose-binding lectin gene and promoter variants in susceptibility to renal infections. Methods: Fifty eight renal recipients' samples which lost their kidneys in result of infection compared with 120 normal controls from Azarbaijan population of Iran. Blood samples were obtained from renal transplant recipients who received renal from March 2004 to July 2005. Mannose-binding lectin genotypes have investigated by polymerase chain reaction and restriction fragment length polymorphism.Results: Allelic and genotypic frequency of the polymorphism at position- 550, -221, +4 and at codon 52, 54 and 57 did not show statistical differences between recipients and controls (P>0.05) but significant frequency of allele B (codon 54) (P=0.02) and Lx haplotype (P=0.002) of promoter was observed in this patients. Conclusion: Our findings provide evidence that presence of different alleles and haplotypes that cause low concentration of mannose-binding lectin in serum is a risk factor for susceptibility to renal infections that cause renal dysfunction.

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THE EVALUATION OF MOLECULAR ASSAY FOR EARLY DETECTION AND IDENTIFICATION OF FUNGAL INFECTION IN SOLID ORGAN TRANSPLANT RECIPIENTS

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Systemic fungal infection has increased in clinical practices along with growing survival of the patients who are immunocompromised and are susceptible to opportunistic fungal infections. So, rapid identification of the species level is necessary for more timely, targeted, and effective antifungal therapy. DNA based methods are more sensitive means of diagnosing fungal infections and have been developed for the purpose of detecting fungal species. DNA amplification with universal fungal primers and species – specific probes has greatly improved the sensitivity test. The aim of the present study was to evaluate a PCR-ELISA method for the early identification of fungal infections. One hundred sixty eight recipients who had undergone transplantations between Sep. 2004 and Jan. 2006 (75 females, 93 males, mean age =34.4 years) were followed for fungal infections for at least 6 month period. All clinical samples were examined by routine methods. Whole blood specimens were collected prospectively once a week and were evaluated for any invasive fungal infections by pan fungal PCR and PCR-Enzyme Link Immunosorbent Assay. In proven and probable recipients for fungal infections, the sensitivity, specificity, positive and negative predictive values by pan fungal PCR-ELISA were 83.3%, 91.7%, 76.9% and 94.3% respectively. Using PCR assay, fungal infections were diagnosed in 14 recipients (8.3%). Infection in the blood was diagnosed 7-70 days with mean of 21.4 days before the appearance of any clinical signs. The etiologic agents were Candida albicans and Aspergillus fumigatus. Considering the above-mentioned findings, we can conclude that the use of PCR-ELISA in transplant recipients improves the ability of early diagnosis of fungal infection.

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COMPARTIVE EVALUATION OF NESTED _PCR AND ENZYME IMMUNO SORBENT ASSAY METHODS IN DETECTION OF CYTOMEGALOVIRUS-DNA IN HEALTHY BLOOD DONORS REFERING TO FARS BLOOD BANK ORGANIZATION

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Introduction & Objective: Human cytomegalovirus (HCMV) infection is most often asymptomatic in immune-competent individuals with lifelong latency. However, infection with CMV is a major cause of disease and death in immune-compromised patient. Transfusion Transmitted HCMV (TT-CMV) can cause serious morbidity and mortality in certain at-risk patient. The availability of rapid, sensitive and valuable surveillance methods allowing the early diagnosis of CMV infection before disease develops is pivotal for this approach. In Iran, ELISA is the most common method used but interpretation of its results is difficult. Therefore, we evaluated the diagnostic value of ELISA between PCR positive and PCR negative groups of healthy blood donor. Materials & Methods: In this cross sectional study during a period of eight months, blood samples were collected from 364 healthy blood donors referring to the blood bank organization of Fars, Shiraz, Iran. IgG and IgM antibodies against HCMV were measured by the enzyme linked immunosorbent assay (ELISA) test. Also, DNA was extracted from 104 buffy coat and 20 serum samples, a nested- PCR was performed to detect HCMV-DNA.The results were compared with those of Elisa test. Results: Of 364 samples, IgG was detected in 360 (98.9%) sera samples and only 16 (4/4%) samples were both IgG and IgM positive. 64 (64%) of buffy coat samples and 8 (40%) of seropositive samples were positive for HCMV-DNA by the nested PCR. None of 4 seronegative samples was detected positive by PCR method. Conclusion: although PCR assays have diagnostic value in the detection of HCMV infection, ELISA is still more useful for screening blood donors. However, PCR method should be evaluated in seronegative blood donors who might be in the window period of HCMV infection.

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TESTICULAR EXPRESSION OF SYCP3, TSGA10, DAZ AND NYD-SP5 MRNA AS MOLECULAR MARKERS FOR SPERMATOGENESIS

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Introduction: Defective spermatogenesis is a frequent observation in men with infertility and azoospermia, but its definite causes in many men are still unknown. It is becoming clearer in recent years that a significant proportion of male infertility is associated with problems in expression of testis specific genes. The stage of spermatogenesis, in which these genes are expressed, is considered to be helpful as a molecular marker for spermatogenesis.Materials & Methods: We investigated mRNA expression of synaptonemal complex protein 3 (SYCP3), testis specific gene A10 (TSGA10), (DAZ) and NYD-SP5 genes in testicular samples of 110 patients with non-obstructive azoospermia. Semi-quantitative nested RT-PCR was performed to find the strength of gene expression. We also evaluated the expression level of above genes using the histopathological scoring of all samples. Results: Testicular mRNA expressions of SYCP3, TSGA10, DAZ and NYD-SP5 were observed in 60.9%, 36.9%, 61.2% and 100% of patients, respectively. The expression levels of SYCP3 and TSGA10 were correlated with the degree of spermatogenic failure, significantly. SYCP3 was expressed in primary spermatocytes and later stages, while TSGA10 was expressed in final stages of spermatogenesis. NYD-SP5 expression was stronger in patients with higher stages, in contrast to DAZ expression which was not observed in patients with Sertoli cell-only syndrome. Conclusion: Expression of SYCP3, TSGA10 and DAZ in specific stages of spermatogenesis can help histopathological findings in patients with azoospermia who undergo testicular biopsy. NYD-SP5 expression in different stages might be explained either by very high expression in germ cells or by its possible role as a Sertoli cell specific gene.

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ONE WSP AND 16SRDNA STRAIN OF WOLBACHIA PIPIENTIS IN IRANIAN AND TUNISIAN PHLEBOTOMUS PAPATASI: IMPLICATIONS FOR LEISHMANIASIS CONTROL

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Individual wild-caught Phlebotomus papatasi from Iran and Tunisia were screened for infections of Wolbachia pipientis by targeting the 16S ribosomal RNA gene of this intracellular α – proteobacterium as well as its major surface protein gene WSP. Only one haplotype was obtained for each gene, from which it is inferred that only one A-group strain of W. pipientis occurs in P. papatasi throughout much of this sandfly's range. This raises the possibility of using just one genetically modified strain of W. pipientis to drive through wild sandfly population's transgenes for intervening in the transmission of L. major by P. papatasi. This genetically modified strain would have to show the cytoplasmic incompatibility (CI) phenotype in P. papatasi, and the flies into which it was introduced would also have to be infected with wild-type strain of W. pipientis if this also provokes CI.

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CYTOKINE NETWORK IN PATIENTS WITH ANTIPHOSPHOLIPID SYNDROME

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The antiphospholipid syndrome (APS) has been the focus of attention of clinicians, as antiphospholipid antibodies (aPL) react with a wide spectrum of antigen determinants leading to different pathologies. The study included 23 patients with gynecologic pathologies (13 aPL-positives; 10 aPL-negatives), 21 patients with systemic lupus erythematosus (SLE) (11 aPL-positives; 10 aPL-negatives), 28 patients with myocardial infarction (13 aPL-positives; 15 aPL-negatives), and 32 healthy controls. The concentration of IL-6, IL-8, IL-10, IL-12, TNF-α, IFN-γ, sIL-2R and CRP in sera was measured with Enzyme-linked immunosorbent assay (ELISA). In aPL-positive and aPL-negative gynecological patients the IL-6, IL-10 and IFN-γ levels were lower, whereas in aPL-positives TNF-α and CRP concentrations were elevated in contrast to aPL-negatives. TNF-α is a leading mediator in aPL-dependent activation of complement, thus elevating the intensity of placental inflammation resulting in fetal death or intrauterine growth retardation. The precise role of cytokines in gynecologic pathologies remains unknown. Extremely high IL-6, IL-8, IL-12, TNF-α, CRP concentrations were detected in SLE aPL-positives, whereas IL-12, IL-6, CRP levels in SLE aPL-negatives were elevated moderately. In SLE aPL-positives IFN-γ was significantly low; this is probably due to less Th-1 involvement in inflammatory response. Increased IL-6, IL-8, IL-12, TNF-α, sIL-2R, CRP levels and decreased IFN-γ levels were detected in aPL-positive and aPL-negative patients with myocardial infarction. Unfavorable outcome of atherosclerotic lesions were characterized not only by aPLs, but also by a glycoprotein-related autoimmune process. These data are indicative of the possibility of cytokine therapy correction in investigated pathologies.

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CATION EXCHANGE CHROMATOGRAPHY: A METHOD FOR THE SEPARATION AND DETECTION OF GLOBIN CHAIN VARIANTS

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There are several point mutations in hemoglobin (Hb) genes which can cause hemoglobinopathies. Since the mutant Hbs do not have any obvious electrical charge, globin chain separation is helpful for diagnosis of unknown Hbs. Therefore, the present study was carried out to detect alpha or beta chain variants by cation exchange chromatography. In this study 31samples having an abnormal Hb were selected. Complete blood cell count (CBC), %HbA2 and % HbF determined by routine methods, and cellulose acetate and citrate agar electrophoresis were performed for all samples. For HbS confirmation, solubility test was performed, and globin chains were separated by carboxymethyl cellulose (CMC) chromatography in the presence of 8 M urea.14 SPSS softwares were used for statistical analysis.According to the obtained results, all of the samples had an abnormal band on cellulose acetate electrophoresis and citrate agar electrophoresis. The CMC chromatography showed that13 patients had abnormal beta chain, 16 patients had abnormal alpha chain, and 2 remaining samples had both abnormal alpha & beta chain. In conclusion CMC chromatography and globin chain separation is a helpful guideline for the selection of an appropriate gene for DNA sequencing. Moreover, this method is useful for screening of hemoglobinopathies and beta thalassemia coexistence in population studies.

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USEFULNESS OF PCR IN THE DIAGNOSIS AND SPECIES IDENTIFICATION OF CUTANEOUS LEISHMANIASIS AGENTS IN MICROSCOPIC NEGATIVE LESION SMEARS

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Background: In the case of negative results from the microscopic examination of stained smears of patients with clinical signs of cutaneous leishmaniasis (CL), diagnosis of

CL will be difficult. In these cases, it is necessary to use specific diagnostic methods such as PCR approaches for the successful treatment of the disease. Methods: Fifty-one suspected patients with CL which leishmania amastigotes have not been observed in their stained smears by microscopy were tested. DNA was extracted from their smears and analyzed by two specific PCR approaches for leishmaniasis diagnosis and leishmania species identification. Using these methods, conserve and variable regions of kinetoplastic DNA of leishmania species have been amplified respectively. Results: PCR results were positive in 37 out of 51 (72.5 %) cases whose further direct microscopy revealed leishmania amastigotes in only 3 of them (5.9%). None of the samples belong to other dermal diseases showed positive results (specifity 100 %). Using species-specific primers, twenty samples had leishmania major and nine had leishmania tropica. The number, location and duration of lesions were variable. Conclusions: The results showed that KDNA PCR methods have a higher sensitivity against microscopy; furthermore they could identify the parasites species for specific therapies. Microscopy has a low sensitivity in chronic and unusual CL cases.

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AN IRANIAN CHILD WITH HBQ-IRAN [α75 (EF4) ASP:HIS] /-α3.7KB/ IVSII.1 G→A: FIRST REPORT

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HbQ-Iran [α75 (EF4) Asp: His] is an α-chain variant that in the heterozygous state has normal hematology and has not been reported in association with a thalassemic phenotype. Here, for the first time, we describe the hematological characteristics of a 5 year old boy with HbQ-Iran /-α3.7kb trans to HbQ-Iran mutation /β0-thalassemia (IVSII.1.G→A) living in the Kermanshah province of Iran. The level of HbQ-Iran was found to be 22.4%. However, a significant reduction in MCV (59.3 fL) and MCH (19.6 pg) and an elevation of HbF (6.3%) was observed.This report indicates that HbQ-Iran is a benign structural variant of Hb, that in combination with -α3.7kb gene and β0-thalassemia, presents a minor β-thalassemia picture with moderate anemia.

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MOLECULAR ANALYSIS OF 40 THALASSEMIA INTERMEDIA PATIENTS IN TEHRAN

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Thalassemia intermedia is a clinical definition used to describe thalassemic patients who have less severe phenotype than thalassemia major. Genetically, it is very diverse and there is many factors influencing the severity of the disease. 40 β thalassemia intermedia patients were selected and genomic DNA was extracted from 5-10 ml of peripheral blood by salting out method. The amplification refractory mutation system (ARMS)-PCR was used to detect the most prevalent β thalassemia allele, i.e. IVSII-I (G>A). Direct sequencing was performed on the samples which had at least one normal allele in order to detect the β-globin gene mutations. Furthermore, the analysis of XmnI polymorphism was done by PCR amplification and the enzymatic digestion of PCR product. Of the 80 alleles being analyzed, the IVSII-I was the most prevalent with 40 allele frequency, followed by CD8 (8), CD30 (6), FSC36/37(3), IVSI-6(2), CD15 (2), FSC8/9(2). IVSI-110, FSC83, -88, CD5, IVSI-5, del 22/23/24, IVSI-25del and CD90 had one allele frequency. The XmnI haplotype analysis showed that the majority of alleles (77.5%) were positive for this restriction site. β0 alleles were the predominant mutations in this cohort. So, the other causes of thalassemia intermedia like co-existence of α- thalassemia or genetic determinants causing high HbF synthesis should be investigated. Probably there is extra α globin genes (ααα or αααα) or other unknown factors in the heterozygotes who presented with clinical phenotype of thalassemia intermedia.

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PROTEIN PROFILE CONSIDERATION OF URMIA LAKE MODERATE HALOPHILIC BACTERIA CULTURED IN DIFFERENT NACL CONCENTRATION, MEDIA CULTURES

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Our country Iran is located in droughty region. So it is important to study the mechanisms used by microorganisms and local plants for tolerating the extreme conditions of their habitat. Identifying the gene pool of these organisms and transferring the useful sequences to strategic agricultural products will lead to provide economical progress. One of the ways to obtaining this gene pool is identifying and isolating osmoprotector proteins. To achieve this goal we studied the halotolerance ability from various species of moderate halophilic bacteria isolated from Urmia Lake water. We selected individual specie of the bacteria. Then we cultured the bacteria in variable salt concentration, nutrient medias with different series of NaCl concentrations (2.5 to 20 percents NaCl dissolved). Then we extracted proteins of bacterial membranes by enzymatic, chemical and physical methods. Thereafter we provided a protein profile study of isolated bacterial proteins from each individual concentration series of salty medias, by SDS polyacrylamide gele electrophoresis method (SDS PAGE). In the electrophoretic results we detected an outstanding protein band with approximately 18000 Daltons molecular weight which appears in protein profiles prepared from 10 to 20 percents of NaCl media cultures. We can say this protein band contains complete or fragments of osmoprotector proteins or enzymes which cause

osmotolerance ability in this microorganisms. Afterwards we examined the molecular characteristic features of the distinct protein band contents, by HPLC and isoelectro focusing methods.

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GALECTIN-3, HBME-1, FAS AND FASL IN THE PREOPERATIVE EVALUATION OF THYROID NODULES

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Growth of thyroid pathology has been observed in many world countries. This problem has become especially actual in Russia and neighbor countries after the Chernobyl AES accident. However, an increase of thyroid size and the appearance of nodules can be due not only to malignant cell transformation, but also to actions of environmental factors or hereditary mutations. Therefore, a very important task is to differentiate preoperatively the type of pathology in thyroid nodules (thyroiditis, benign or malignant proliferation). This will allow ruling out surgeries that at present are often performed without the appropriate necessity. Solution of this problem and a fast and effective diagnostic approach can become a combination of fine needle aspiration with detection of specific marker proteins of thyroid tumor growth by means of immunocytochemical staining. The goal of the present work was to study efficiency of using antibodies to marker proteins HBME-1, galectin-3, Fas, and FasL for differential diagnostics of thyroid pathology. Galectin-3 belongs to the lectin family, participates in interactions between cells and cell matrix, and is used as a marker for development of neoplastic processes. HBME-1, an antigen of mesothelial cells, has been successfully used in several hospitals for differential diagnostics of various forms of malignant thyroid tumors. The Fas and FasL system of transduction of apoptotic processes can play an important role in progression of tumor growth. Using this approach, we studied a group of patients (n = 50) admitted for examination with suspicion for thyroid cancer. Morphological assay revealed cases of such pathologies as autoimmune thyroiditis, nontoxic adenomatous goiter, papillary cancer, follicular cancer, and Hürthle cell adenoma. The tumor growth marker proteins stained with FITC were revealed by means of indirect immunofluorescence with use of confocal microscope. The character of fluorescence was different in different samples. Usually we could observe individual fluorescent cells or focal clusters of such cells. We have revealed fluorescence of marker proteins in 50% of the patients with diagnosed tumor pathologies. The fluorescence was also detected in some patients with non-tumor pathology. The highest detectability was found for papillary cancers. As a rule, expression of galectin-3 and Fas was revealed in all these cases, whereas the presence of HBME-1 was the least frequent. We consider it is useful to detect such marker proteins as galectin-3 and Fas for help in determination of the malignant character of thyroid pathology. Besides, it is necessary to continue observation of patients of this group in order to correct diagnoses made out by morphological and immunocytochemical criteria.

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THE DIAGNOSIS OF TRANSLOCATIONS IN PATIENTS SUFFERING LEUKEMIA BY MULTIPLEX-RT-PCR

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The word leukemia is applied for describing a great number of hematopoetic cancers in bone marrow. Acute lymphoblast leukemia (ALL) is a particular case of blood cancer which includes 75 percent of leukemia cases in childhood. Universal statistics shows that this kind of cancer has been developing to 27 percents in the children under 15. The researchers believe that the genetically and environmental effects are the fundamental cause in appearing this disease in infants. The number of children suffering from blood cancer who consult Shafa hospital (which is the only center of cancer in Khuzestan province) is over the universal standard. Therefore improving the management of remedy of patients should be more taken in to consideration. The use of repellent poisons of vegetable pests in the extreme, the repelling of industrial sewage of factories in an unhealthy way, malnutrition in infants and abundance of viral contagious diseases are the probable causes of prevalence of blood cancer in children in Khuzestan province. Chromosomal abnormality, specially the replacement of chromosomal material (Translocation) is one of the main reasons in producing leukemia. The kind of translocation plays a key role in managing the remedy. For instance translocation t (1;19) which is often accompanied by the counting of the high leukocyte and the increased LDH shows better consequence in high and hard Chemotherapy. The aim of this research is to attain a reliable, rapid method with low expense in diagnosing the translocations which are the main causes of appearing these kinds of cancers in different types of leukemia. The molecular method Multiplex-RT-PCR proved that it is possible to diagnose 4 common translocations consist of [t (1;19), t (9;22), t (12;21), t (4;11)] immediately. Translocation t (9;22) is the most common kind of chromosomal replacement found among 50 patients. It was proved in a clinical diagnosis that more than ¼ of these patients suffer from acute lymphoblast leukemia (ALL). Translocations t (12;21) and t (1;19) come in next steps respectively. The translocation t (4;11) was seen only in one patient suffering from ALL. On the other hand it was not observed in any of the 8 kinds of translocation lymphoma.

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DESIGN AND DEVELOPMENT OF MULTIPLEX NASBA TECHNIQUE FOR SIMULTANEOUS DETECTION OF HCV AND HIV-1

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HCV and HIV-1 are the two most important infectious agents transmitted by blood transfusion and their co-infection is a relatively common clinical occurrence affecting approximately 25% of HIV-1 and 10% of HCV infected individuals. Serological tests are commonly being used for the detection of these two viruses, which do not have sufficient efficiency especially during the window period. A more reliable and sensitive method called multiplex NASBA assay was developed for this purpose. It is a continuous, isothermal process based on amplification of RNA. Reverse transcriptase, RNase H, and T7 RNA polymerase are the enzymes simultaneously used in this reaction plus two primers with a T7 promoter sequence at the 5' end of one primer. In this study two specific primer sets were designed for the conserved regions of HIV-1 pol and HCV 5' NCR. Both viral genomes were identified by the distinctive size of amplicons, which are 155 and 248 bases respectively. These sequences were also cloned in T/A clone vector for the internal control. To confirm the sensitively and specificity of the test several blood samples of co-infected patients and normal donors were assayed. Undoubtedly, this method represents a useful alternative for the detection of HIV-1/HCV co-infection, reliable for a rapid, sensitive and relatively inexpensive isothermal screening of blood donors.

p-409
MUTATION SCREENING OF APC GENE IN FAP PATIENTS BY A NOVEL CSGE METHOD

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Introduction**:** Familial adenomatous polyposis (FAP) is an autosomal dominantly inherited colon cancer with high penetrance, characterized by more than 100 adenomatous polyps in the colon and rectum. Additional features may include desmoids tumors, polyps in the upper gastrointestinal tract, osteomas and congenital hypertrophy of the retinal pigment epithelium (CHRPE). A mutation in APC is found in the majority of cases. Mutation detection and genetic analysis of APC in this syndrome is highly recommended as the penetrance is 100% by 40 years of age. The APC gene has 15 exons and an ORF with 8538 nucleotides which codes a protein with 2843 amino acids. Most of the mutations (85%) in APC gene are situated in MCR (Mutation Cluster Region). Materials & Methods: patients were selected according to accepted diagnosis criteria of FAP. A novel CSGE (Conformation Sensitive Gel Electrophoresis) technique for the first time was set up to screen mutations in this gene. Direct sequencing was used as gold standard to confirm CSGE results. Results: CSGE analysis showed mutations in all 10 patients. Further analysis by sequencing confirmed CSGE results. Conclusion: Conformation Sensitive Gel Electrophoresis can be used as a simple, cost effective and sensitive mutation screening method. Our analysis was in favor of a two step genetic analysis protocol in which a simple screening test is completed by sequencing to lower test cost without losing sensitivity.

O-410
UTILITY OF CAMEL SUBCLASS ANTIBODIES IN EGFRVIII ENZYME-LINKED IMMUNOSORBENT ASSAY

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Serum of Camelidae contains three subclass immunoglobulins: IgG1, IgG2 and IgG3. IgG1 is a conventional heterotetramer antibody composed of two light and two heavy chains while two other subclasses lack light chains and CH1 domain and named as Heavy Chain (HC) antibodies. Although genetic manipulation on HCAbs and synthesis of their variable domain, VHH, for immunotherapeutic purposes are comprehensively considered, their efficiency in diagnostic methods like ELISA is not already evaluated. In the present study, anti EGFRvIII antibodies were purified from the serum of an immunized camelus dromedarius using ammonium sulfate precipitation and immunoaffinity chromatography. The three subclasses of camel IgG were separated and purified following protein G column chromatography. SDS-PAGE was performed to investigate the efficiency of purification steps. In the same time, in order to obtain required antigen, the cell line HC220d2/c was cultivated in DMEM culture medium, lysed following Triton-x100 treatment and centrifuged. The antigen was purified from supernatant using a concanavalin a column chromatography. Finally despite of remarkable function of total camel antibody the results in the case of utility of fractionated subclasses in both of ELISA systems which HRP or Penicillinase acted as tracer, was not satisfactory. The affinity constants of total IgG, IgG1, IgG2 and IgG3 was found to be 4.48×107, 1.3×107, 106×4.23 and 106×1.67 M-1 respectively. This is the first report of application of camel subclass antibodies in Enzyme-linked immunosorbent assay.

p-411
AN IMPROVED PCR-BASED AMPLIFICATION OF UNKNOWN HOMOLOGOUS DNA SEQUENCES

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PCR is a popular molecular research tool with a wide application nowadays. Among its most prevalent applications is amplification of cognate genes by primers designed based on the limited available amino acid or sequence homology information and elucidation of the evolutionary relationships and phylogenetic analysis of the homologous sequences (e.g. gene family members). The PCR primers used for cloning of evolutionary conserved genes or homologous DNA

sequences are usually guessmer oligonucleotides. Although some degree of mismatches between the primers and the original DNA template can be tolerated but, the position of these mismatches is critical to successful PCR.DNA polymerases fail to initiate polymerization, when primers are completely matched to the template but with a single mismatch at their 3'-ends.We introduce a simple way using Pfu polymerase to overcome possible PCR amplification failure because of 3'-end mismatches of guessed primers with the target DNA.

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SURVEY OF A AND B ESTERASE ENZYMES IN THE DELTAMETHRIN RESISTANCE IN CULEX TRITAENIORHYNCHUS (DIPTERA: CULICIDAE) AS VECTOR OF JAPANESE ENCEPHALITIS

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Synthetic pyrethroids are now being used as alternative insecticides to vectors control in many parts of world. However, continuous and indiscriminate uses of these insecticides also lead to the development of resistance in insect pests. Better understanding of the biochemical mechanism involved in the resistance of the brain fever (Japanese Encephalitis) vector could subsequently be used to detect the development of pyrethroid resistance and reason for it in the field. Field collected Cx. tritaeniorhynchus larvae were colonized in the laboratory for 12 generations and acclimatized. An isofemale line was raised from this colony and the larvae were subjected to continuous deltamethrin selection pressure. LC50 and LC90 values were calculated at every generation the values indicated that at the end of ninth generation, the larvae have developed 85 fold tolerances in terms of LC50 value compared with the first generation. The reason for this kind of resistance was analyzed on the basis of differential activity of A-esterase and B-esterase. A significant correlation ($P<0.05$) was observed between B-esterase activity with the rise in the LC50 and LC90 values. The isozyme analyses of the A-esterase and B- esterase using poly acrylamide gel electrophoresis (PAGE) have shown differential profiles.

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CYTOCHROME C OXIDASE EXPRESSION LEVEL IN WHITE BLOOD CELL OF PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Chronic obstructive pulmonary disease (COPD), a lung disease which is characterized by decreased expiratory flow rates, increases pulmonary resistance and hyperinflation. In vitro studies indicate that the activity of Cytochrome C Oxidase (COX) can be directly regulated by the presence of molecular oxygen. Thus, a better understanding of functional pattern of COX in patients with COPD in comparison with normal subject can provide an important link between the availability of oxygen to tissues and amount of oxygen uptake and energy production in these patients. We studied 42 patients under clinically stable conditions and 50 healthy sedentary volunteers of similar age. Whole blood was collected and white blood cells (WBCs) separated and lysed. The homogenates were centrifuged and the supernatants were used for total protein content, COX and citrate synthase activity. Absolute specific COX activity and relative activities were determined. After isolation of RNA and cDNA synthesis, expression level of mitochondrial subunits of COX evaluated by two step real time RT-PCR method.Mitochondrial COX activity and specific activity (absolute & relative) in WBCs were significantly increased in patients with COPD in comparison with control samples ($p< 0.05$). Expression level had a similar pattern of increase. These results indicate that the activity of COX increased in WBCs of patients is due to rising in expression level not in rate of enzyme activity. However, it is not clear that this is a primary or secondary effect of hypoxic condition in these patients and need further investigation.

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COMPARISON OF MDCK CELL CULTURE AND RT-PCR METHOD OF HUMAN INFLUENZA VIRUS

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Introduction & objectives: MDCK cell line is used for isolation of influenza virus in most of laboratory, but RT-PCR is effective for the initial diagnosis and has greater sensitivity than other available rapid assay. Molecular assay also can be used for subtype influenza isolation. In this study we compared MDCK cell culture and RT-PCR method on the circulating influenza virus in Tehran. Materials and Methods: Nasal and pharyngeal swabs were transported to the lab, in transport media. MDCK cell culture was inoculated with the samples. Fallowing incubation, culture media was assayed for HA.RNA was extracted from suspension of cell culture and fallowed by RT-PCR using specific primers for influenza virus typing and subtyping. 10 percent of the amplified product was loaded in an agarose gel containing 1µl ethidium bromide.Electrophoresis was conducted in TBE buffer and visualized by UV transillumination. Results: In this study, we displayed most of patients' samples that were negative in MDCK cell culture, were positive in RT-PCR. From total number of 50 samples, 5 samples were isolated with cell culture, 12 samples were isolated with RT-PCR method that confirmed isolated samples from KDCK culture.

Bioinformatics

p-415
SIMULATION AND IN SILICO STUDY OF TRUNCATED FORMS OF P16INK4A INTERACTION WITH CDK4

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The prediction of protein-protein interactions can greatly increase the structural and functional information pertaining to biologically active complexes. The information gathered from such studies can lead to designing and application of new modification strategies in order to obtain a desired bioactivity. Many application packages and servers performing docking, such as HEX, DOT, AUTODOCK, ZDOCK, etc. are now available for predicting the lowest free energy state of a protein complex. We studied interaction of two proteins: Cyclin-dependent kinase 4 (CDK4), a key molecule in the regulation of cell cycle progression at the G1-S phase restriction point and p16INK4a, a tumor suppressor which inhibits CDK4 activity. Considering the more critical regions of p16 interaction, we have created seven truncated structures including P16-A (lacking the 76 C-terminal amino acid), P16-B (lacking the 79 N-terminal A. A), P16-C (lacking the 41 N-terminal A. A), P16-D (lacking the 109 C-terminal A. A), P16-E (lacking the 66 N-terminal A. A), P16-F (lacking the 66 N-terminal and 42 C-terminal A. A) and P16-G (lacking 42 N-terminal and 53 C-terminal A. A). The tertiary structures have been determined by ProSAL, GENO3D Web Server. We have evaluated their interactions with Cdk4 using two docking systems, HEX 4.5, and DOT 1.0 Beta. Calculations have been performed on a high-speed computer. Based on the obtained results, p16-A, p16-B and p16-C have shown the best fit complexes with the lowest minimized energy among the truncated p16 forms. The free energies were compatible with that of p16 wild type.

p-416
BIOINFORMATIC ANALYSIS OF SNPS OF SCHIZOPHRENIA CANDIDATE GENES, INTERFERING WITH NORMAL SPLICING PROCESS

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Despite the ability of genetic association studies in assigning schizophrenia susceptibility genes, results have been quite spurious. SNPs (single nucleotide polymorphisms) are the most common genetic variation in schizophrenia association studies. Most of these variations are located in noncoding part of genome, previously thought to have no particular function. In recent years regulatory function of these SNPs has attracted much attention. SNPs which are located on regulatory elements might interfere with mechanisms that regulate gene expression such as splicing. Exonic Splicing Enhancers (ESEs) are one of the most important splicing regulatory elements which can stimulate splicing and seem to be particularly relevant for regulating alternative splicing. ESEs appear to be very prevalent and may be present in most exons, if not all. We have computationally analyzed SNPs of 16 candidate genes for schizophrenia, to find whether they interfere with the normal function of ESE. In total, 9335 SNPs were analyzed by S/R rich protein binding site prediction algorithm. 1.12% of SNPs can potentially interfere with ESE function. These SNPs might have regulatory function thus are interesting candidates for schizophrenia association study.

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NOVEL HYBRID MODELING PROCEDURE IN PREDICTION OF METASTASIS IN ADVANCED COLORECTAL CARCINOMAS USING COMPARATIVE GENOMIC HYBRIDIZATION DATA

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Logistic regression model (LRM) and artificial neural networks (ANNs) as two non-linear models have been used to establish a novel two-stage hybrid modeling procedure for prediction of metastasis in advanced colorectal carcinomas. The training set included comparative genomic hybridization (CGH) data from 54 specimens. The LRM was used at the first stage of hybrid modeling procedure to evaluate the contribution of DNA sequence copy number aberrations detected by CGH in determining the metastasis in advanced colorectal carcinomas. Then, the most effective parameters were selected by a LRM. Selected effective parameters in the LRM were gain of 20q11.2, loss of 1q42, loss of 13q34, gain of 5q12, gain of 17p13, loss of 2q22, loss of 11q24 and gain of 2p11.2, in order of importance among 565 detected chromosomal gains and losses. Consequently a neural network model was constructed and fed by the parameters selected by LRM to build a hybrid predictor. In this study, self-consistency and jackknife tests on a database containing 54 specimens were used to verify the performance of the hybrid model. The results showed that our two-stage hybrid model approach is very promising and may play a helpful role in selection of appropriate protocols for treatment of colorectal carcinoma.

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MULTIFRACTAL ANALYSIS OF CHAOS GAME REPRESENTATION IMAGES OF MITOCHONDRIAL DNA

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Chaos Game Representation (CGR) is an interactive mapping method which can convert a nucleotide sequence into a unique and scale-independent image. In CGR image of a DNA sequence both global and local patterns are displayed. These images allow us to investigate hidden self-similar patterns in genomic sequences. In this paper the fractal properties of these images have been quantified by fractal dimension calculation and fractal quantitative characteristics of CGR images have been considered by multifractal analysis to evaluate the possible evolutionary properties of the CGR images of mitochondrial DNA (mtDNA) in 10 different species. The multifractal spectra of CGR images of mtDNA sequences revealed that there is some evolutionary information in them which is applicable in phylogenetic studies. Further studies can help to establish a novel hypothesis on the application of fractal dimensions of CGR images of mtDNA for reconstructing phylogeny.

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THREE DIMENSIONAL CHAOS GAME REPRESENTATION OF GENOMIC SEQUENCES: A NOVEL VISUALIZATION METHOD

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Chaos Game Representation (CGR) is a method for converting a genomic sequence into a two dimensional scale-independent and unique image. In two dimensional CGR images, the mapping equations result in an image limited in a square. These images show a local and global view of the whole genome and have potential applications for whole genome analysis. In this paper a novel algorithm is introduced which makes it possible to convert a genomic sequence into a three dimensional image based on chaos game mapping equations. In fact in this procedure the mapping equations will result in a three dimensional image limited in a cube. The algorithm for three dimensional chaos games (8 points) has been coded in Matlab 7.0 language and different genomic sequences have been used to produce and compare three dimensional chaos game representation images. This new algorithm and its implementations have been compared with other methods and the possible applications have been discussed.

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HOMOLOGY MODELING OF HUMAN MALTASE ENZYME AND IN SILICO CHARACTERIZATION OF ITS ACTIVE SITE

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Type II diabetes mellitus is currently recognized as a growing threat to global public health. Amongst its potential medications, alpha-glucosidase inhibitors are remarkable due to their effect on the reduction of post-prandial hyperglycaemia, as the result of inhibiting the final step in dietary carbohydrates digestion. Study on the interaction of inhibitors with alpha-glucosidase at the molecular level can lead to a better understanding of the inhibition mechanism. Since there is no available crystal structure for this enzyme, using a model enzyme obtained by computational methods could be helpful for designing potential novel inhibitors. In the present study, homology models of the maltase enzyme monomer were generated and subsequently refined with MODELLER 8v2 software, using the alpha-glucosidase structure of *Sulfolobus solfataricus* as a template. Model quality evaluation programs PROCHECK 3.4.4 and ERRAT 2.0 were used to choose the best structure, on which SCWRL 3 program was applied as a final step to obtain alternative side chain positions. The final model of secondary structure was found to be β (α/β) β sandwich motif. A high degree of conservation was observed between the model active site and its template. In order to find suitable binding positions for the inhibitors molecular interaction fields were generated with probes related to known ligands such as maltose, acarbose, and voglibose using GRID 22b for both structures in the vicinity of the active site.

p-421
MODELING OF INHIBITORS BINDING TO DEOXYGUANOSINE KINASE

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Human deoxyguanosine kinase (dGK) (EC 2.7.1.113) is a mitochondrial enzyme that catalyzing the first steps in the salvage pathway of deoxynucleosides biosynthesis: Deoxyguanosine +ATP dGMP + ADP dGK can also use UTP or inorganic polyphosphate as phosphate donors instead of ATP. dGK is a dimeric enzyme with 10 alpha helices and 4 beta strands in its secondary structure. Nucleotide analogous of deoxyguanosine and ATP can act as enzyme inhibitors. dGMP, dGDP, dGTP, dIMP, dITP, dTMP and dTDP were shown to act as enzyme inhibitors and their inhibitory constant (Ki) were reported previously. By using the docking (Hex 4.2) and molecular dynamic method we attempt to simulate the enzyme structure in aqueous condition and optimized it for more stable conformations. After that we docked the inhibitors against the optimized enzyme. The best docking solutions were optimized by using a molecular dynamic method of hyperchem7 software. Our result shows that the enzyme inhibitors bind to enzyme through two kind of binding sites. The first one is the enzyme active site or competitive binding site and the other is the postulated inhibitory binding site or noncompetitive binding site. We found that the later binding site includes number 1 and 10 helices. In each class of

inhibitors the inhibitory potency (low Ki) is completely interpretable by the shape matching and docking energy which include the electrostatic and hydrogen binding energy and also by the conformational changes after the binding of inhibitors. We think this attempt may introduce an easy and fast way to study the conformational changes in the field of protein ligand binding in biochemistry.

p-422
DESK-TOP ANALYSIS OF NON-CODING REGIONS OF THE HUMAN FACTOR VIII GENE TO IDENTIFY CANDIDATE REGULATORY ELEMENTS

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To identify possible regulatory elements in the intronic regions of human coagulation factor VIII gene, the non-coding sequences of this gene were compared to the parts of human factor IX intron-1 sequences whose regulatory functions had been suggested previously. This comparative study has resulted in detection of several repeated sequences within hFVIII intronic and UTR regions, varying in size from 50 to 350 base pairs with more than 74% homology. It seems that the distribution pattern and frequency of repeated elements among hFVIII introns, especially in first and last introns and within each intron are nonrandom. This distribution in some of the introns may lead to formation of stem-loop structures that might be effective in the stability of the hFVIII mRNA precursor. More analysis shows that the detected elements belong to sub-families of retrotransposon, known as Alu sequences. The sequence analysis of the repeated elements indicates the presence of many short sequences with the potential transcription-factor binding activity. This finding supports the possible regulatory function of the repeated elements found in the hFVIII intronic regions. The data provided in this study including the similarity of the intronic regions of hFVIII gene to the parts of intron-1 from hFIX gene, which had been proposed for some candidate regulatory functions, and the non-random distribution of the repeated elements within hFVIII intronic regions has provided convincing evidences for the presence of some regulatory elements in non-coding regions of hFVIII gene. To use as regulatory elements further experimental data are required to confirm the functions of these Alu-containing fragments.

p-423
MONTE CARLO SIMULATION OF THERMODYNAMIC STUDY FOR UNUSUAL AMINO ACIDS OF LANTIBIOTIC PEPTIDES IN WATER

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Lantibiotics are a major group of peptide antibiotics such as nisin and subtilin involve inpost-translational modifications, including dehydration of serines and threonines, formation of thioether cross-linkages. The lantibiotics offer chemical, physical, and hence biological properties that are not attainable by polypeptides that lack these modified residues. For example, the dehydro residues (residues dehydrobutyrine (Dhb), dehydroalanine (Dha), lanthionine, and b-methyl lanthionine) are electrophiles, whereas none of the ordinary amino acids is electrophilic. To ascertain the role of specific amino acid residues in antimicrobial activity, we have studied the interaction between these residues and water solvent molecules which has an important effect in various biochemical processes. We have calculated total energy and free energy of the solvation of these residues in water in different temperatures by Monte Carlo simulation. Monte Carlo sampling temperatures have been determined and ranged from 300-373°K systems. These residues first optimized in the gas phase and then placed in a cubic box of water. The computations have shown that Dha has the highest value of solvation free energy. These results confirmed that this group of residue exhibits the highest stability at high temperature. This is particularly important because of increasing antibiotic resistance observed among microbial populations due to the widespread use of existing peptide antibiotics by peptide engineering in future. Therefore, it is desirable for production of the lantibiotic forms from bacteria to take advantage of developments in food preservation.

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MECHANISM OF PROTON TRANSFER IN AQUAPORIN CHANNEL: A MOLECULAR DYNAMIC SIMULATION

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Aquaporins are membrane water channels that play critical role in controlling the water contents of cells. In nature, aquaporin rapidly filters water through membranes. More than ten different aquaporins have been found in human, and several diseases, such as congenital cataracts and nephrogenic diabetes insipidus, are connected to the impaired function of these channels. Aqp4 is constitutively expressed in the brain predominantly in astrocyte cell. In this study, we have used molecular dynamics simulations to solve a biological mystery: how water molecules can pass through a protein pore in a cell membrane as rapidly as they do without ferrying extra protons across with them. Water permeation and electrostatic interactions between water and channel are investigated in the Aqp4, a member of the aquaporin water channel family, by Charmm software. In our study, on the regulation and detailed mechanism of action AQP4 was considered at DMPC membrane. The simulation reveals a delicately choreographed dance of the water molecules, directed by carefully positioned amino acid residues throughout the channel interior. Water molecules passing the channel are forced, by the protein's electrostatic forces, to flip at the center of the channel. Therefore, breaking the alternative donor-acceptor arrangement is necessary for proton translocation. These results show the improved undressing of water permission in membrane. We hope our model simulation could be useful for synthesis of functional inhibitor antibiotics for this group of water channel in future.

p-425
RELATIVE ENTROPY CALCULATION FOR DIFFERENT POSITIONS OF AMINO ACIDS IN BETA STRANDS

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Amino acids seem to have specific preferences for various locations in beta strands; this is previously seen in alpha helices. For examination of this issue, we selected protein chains with sequence similarity less than 25%. The protein structures had been determined with x-ray crystallography with a resolution of 2.5 angstrom or better in which each of them belonged to a super family. Beta strands along with the two amino acids preceding and ending the strands in protein chains were extracted from the whole dataset. We used the Relative Entropy formula in order to calculate preferences of amino acids in different single positions: Where Q represents the background, relative entropy represents information of P. Relative entropy of an amino acid ai at a given position Nj of the beta strand with respect to the background is given by: Where p (aiNj) represents the probability of occurrence of amino acid ai in the position Nj of the beta strand and q (ai) represent the background probability of occurrence of the amino acid ai in the database of beta strands. We defined the relative entropy for the location Nj of an beta strand as the sum of the relative entropies of all amino acids occurring in that position, given by: Our results show that the positions of N'', N', C' and C'' which are two positions before N-terminal and two positions after C-terminal, occupy polar amino acids abundantly, i.e. polar amino acids show a relatively high tendency for these positions and N1, N2, N3, M, C3, C2, and C1 positions occupy non-polar amino acids in beta strands i.e. non-polar amino acids show a high tendency for these positions.

p-426
T AND B CELL EPITOPES PREDICTION OF IRANIAN SAFFRON (CROCUS SATIVUS) PROFILIN BY BIOINFORMATICS TOOLS

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Profilins are a group of ubiquitous actin monomer binding proteins that are responsible for regulating the normal distribution of filamentous actin networks in eukaryotic cells. Plant profilins form a well-known panallergen family responsible for cross-sensitization between plant foods and pollens. We sought to map T and B cell epitopes on the *Crocus sativus* profilin by bioinformatics tools. For prediction of T cell potential epitopes we used IEDB (Immune Epitope Database) server which uses a partial least squares-based multivariate robust statistical approach to the quantitative prediction of peptide binding to major histocompatibility complexes. In the case of B cell sequential epitopes BepiPred method was utilized in which the hidden Markov model (HMM) was combined with Parker propensity scale method. Among all MHC alleles, the results from DRB1 0101, DRB1 0701 and DRB1 0901 show significantly lower IC50 than other alleles. The 19-VLTSAAILG-27 corresponding to DRB1 0101 allele is the peptide with the best binding affinity. Through this study four potential linear B cell epitopes were calculated which are I: 31- SVWAQSAGFPELKPA-45, II: 54- FNEPGSLAP-62, III: 80-GVVIRGKKGSGGVTI-94 and IV: 108- EPMTPG-113. Profilin has been identified as an important cross-reactive allergen for patients suffering from multivalent type I allergy. So the determined peptides are useful for vaccine development because they can reduce the time and minimize the total number of required tests to find the possible proper epitopes.

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PREDICTION OF PHOTORHABDUS LUMINESCENS LUCIFERASE SUBUNITS INTERACTIONS THROUGH STRUCTURAL BIOINFORMATICS

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Bacterial luciferases are heteropolymeric enzymes consisting of two non-identical subunits (alpha and beta) that catalyze the oxidation of a long-chain aldehyde and release energy in the form of visible light. Identifying critical residues and their binding sites between two subunits is essential to designing new luciferases. Here we present a new modeling and docking studies of *Photorhabdus luminescens* luciferase using structural bioinformatics servers. A homology modeling of alpha and beta subunits in bacterial luciferase obtained from the P. luminescens based on the crystal structure of Vibrio harvi luciferase obtained from protein data bank (PDB code 1BRL). With this model a protein-protein docking study have been performed and the results will been reported.

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AN IN SILICO STUDY ON THE INHIBITOR SELECTIVITY OF CATHEPSIN G

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Inhibitors of cathepsin G, a serine protease secreted by neutrophils have been shown to have anti inflammatory properties in vivo. One of the first non peptidic inhibitors of cathepsin G1 has also an excellent inhibitory effect on chymase, a serine protease derived from mast cells and involved in inflammatory diseases. While this dual inhibitory property might be important regarding its use as a medicinal agent against asthma, there is still the potential of side effects as a result of undesired interaction of the compound with other enzymes possessing similar active site. In this study, the BLAST tool was used in order to find similar proteins to

cathepsin G, both against PDB and swissprot databases. A selection of enzymes with more than 50% similarity and preferably not involved in pathological inflammatory conditions, were primarily chosen and aligned in 3D using the identity or blosum62 substitution matrices within the homology module of MOE 2006.08, in order to reach maximal superposition of the catalytic residues. Molecular interaction field computation was then carried out with GRID 22b, using hydrophobic probes and also carbonyl, nitrogen and phosphate probes to match structural features of the ligand. The resulting fields were observed and compared with GVIEW 2.0 and BIOCUBE v.1.00 in order to find possible differences and comparison was made between these results and the existing reported inhibitor activity of the ligand on these enzymes.

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COMPUTER-AIDED ANALYSIS OF AMINO ACID SEQUENCES FROM PHENYLALANINE DEHYDROGENASE ENZYMES

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NAD^+-dependent Phenylalanine dehydrogenase (PheDH; EC 1.4.1.20) catalyzes the reversible oxidative deamination reaction of L-phenylalanine to phenylpyruvate. It has received considerable attention for clinical diagnosis of phenylketonuria (PKU) and synthesis of pharmaceutical intermediates as well. Understanding of the basis of substrate specificity is very interesting in that it will lead to engineer novel efficient biocatalysts. In this study, amino acid sequences of PheDHs from various sources including bacteria, fungi and single cell eukaryotes were obtained from Swiss-Prot database. These sequences were compared using CLUSTALW software and multiple sequence alignments were done. A homology-based modeling using homologous enzymes *Bacillus sphaericus* PheDH, *Clostridium symbiosum* glutamate dehydrogenase and B.sphaericus leucine dehydrogenase was investigated. Our data revealed that the overall sequence homology between these enzymes is less than 20%. Nevertheless, the molecular modeling based on the sequences alignment suggested that the active sites have similar conservative sequences. These sequences were [LIV] - X (2) - G - G - [SAG] - K - X - [GV] - X (3) - [DNST] - [PL] that created the correct three dimensional structures. In fact, they were very important to determine the differential specificity among PheDHs toward aromatic amino acid substrates. Collectively, this research demonstrated the usefulness of sequence alignment and homology-based modeling to conduct a precise and successful site-directed mutagenesis.

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MODELLING OF 3D STRUCTURE OF HUMAN EP1 RECEPTOR AIDED BY MOLECULAR SIMULATION AND LIGAND DOCKING

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Prostanoids are the cyclooxygenase metabolites of arachidonic acid and presumed to play many important roles in a variety of physiological and pathophysiological processes in body. The prostanoid receptors consist of eight types characterized seven-hydrophobic-transmembrane segment architecture typical of G-Protein Coupled Receptors (GPCRs). The EP1 receptor, a prostanoid receptor subtype E1, was originally described as a smooth muscle constrictor, involved in pain sensitization, fever and inflammation. There are selective agonists that bind to the EP1 receptor; however, these agonists also have significant affinity for other receptor subtypes. Therefore, the knowledge of the structural features of EP1 receptor is of very important for the understanding of its function and for its use for drug design. We report here the 3D structure for the human EP1 GPCR predicted by homology modeling method using the X-ray structure of bovine rhodopsin (pdb code: 1U19) as template. From the clustalW alignments, 3D models were obtained automatically using the method implemented in MODELLER version 9.1. The lipid compatibility scores for helix bundles and for individual helices in the generated models calculated using REPIMPS (Reverse-Environment prediction of Integral Membrane Protein Structure) method. We performed molecular dynamics (MD) simulations of the predicted structure of EP1 receptor with GROMACS package. Also to validate this structure we used the Autodock 3.0 flexible ligand docking method to predict the binding affinity of some agonists and antagonists with known potency.

p-431
STUDY OF DISTRIBUTION AND CHARACTERIZATION OF NON-LTR RETROTRANSPOSONS IN THE HUMAN COAGULATION VIII FACTOR GENE

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In human genome, coding sequences are less than 5% while repeated sequences, which most of them are derived from retrotransposons, are more than 50%. Identification and characterization of the elements that are located throughout them would be important in both understanding of possible gene regulatory elements and their application in

biotechnology. With this aim we studied the distribution pattern and characterization of the repeat elements in human coagulation VIII factor gene. Result showed that more than 65% of the length of this gene made up of Interspersed Repeat elements that comprise four classes including non-LTR retrotransposon, DNA transposon, endogenous retrovirus and LTR retrotransposon with descendant frequency. Although the concentrations of non-LTR Retrotransposon elements in first and last introns are more than others, there are still a considerable number of such elements in the several internal introns. There are three subclasses among non-LTR retrotransposons namely L1, SINE and CR1 which have considerable concentrations. Interestingly, the frequencies of them are length independent. Among the four classes, there are only two subclasses of non-LTR retrotransposon in 5'UTR and there aren't any elements of L1, in spite of its high frequency. Moreover, distribution pattern of SINE elements are rather similar to distribution of L1 elements. The distribution pattern, concentration and frequency of repeat elements, especially SINE and L1, throughout noncoding regions are significant. We can conclude that in addition to first and last introns, some others may have elements that may affect gene regulation by mechanisms we can suggest but need to study more.

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RELATIONSHIP AMONG CpG ISLANDS, REPEAT ELEMENTS AND POSSIBLE PROMOTER-LIKE SEQUENCES IN THE HUMAN COAGULATION VIII FACTOR GENE

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Relationship between CpG islands and promoters has already been established. They are located inside and close to approximately 70% of human promoters (40% of mammalian promoters). According to present study, CpG islands concentration within the human coagulation factor VIII gene (hFVIII) varies from less than 20 to over 80%. They are only located in three regions, one in Intron-1 (Int-1) and two in Intron-22 (Int-22) of this gene, instead of being located near the hFVIII gene promoter. The CpG Islands of int-1 are located in a region containing a number of two subclasses of non-LTR retrotransposon including SINE and L1 elements. The CpG islands emplacing within Int-22 are located in a region containing L1 and DNA-transposon elements. There are 5 and 9 promoter-like elements within Int-1 and Int-22, respectively. In Int-22, the nearer promoter /enhancer to CpG, the more LDF, which indicates the possibility of promoter activity in any region, will be observed. The promoter-like sequences detected in the Int-22 may cover a previously reported bidirectional promoter of two nested genes within Int-22 of hFVIII. In the case of Int-1, the presence of L1 elements together with promoter-like sequences as well as CpG islands can be explained by the fact that Int-1 plays an important role in the regulation of this gene. In conclusion, the presence of L1 element close to promoter-like sequences and concentration of CpG Islands on their vicinity, as shown in this study support a possible interaction of repeated elements with gene regulatory system.

p-433
DIABETES AND GENOTYPES OF VARIOUS HUMAN POPULATIONS

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Introduction: Diabetes is a common disease in various human populations and there has been a lot of attempt to cure it. Attention to various genotypes and it's relation to the amount of penetrance and intensity of disease and how to respond to the cure can be effective to cure and prevent. Difference in the person's genome can lead to the difference in the protein structure and molecule function. The base of the person's response to the various elements is related to its genotype. In this research we studied various genotypes of human population and genetic disease and genetic polymorphism and its relationship with the penetrance and intensity and epidemic of diabetes. Methods: We studied all of the single nucleotid polymorphism (SNP) of INSR molecule that is related with diabetes and insulin in the various human populations and various genotypes. After various gene sequence analysis of data by the biologic software (Cn3D,Oligo,..) and database (Blast, PDB, Genecards,..) and use of bioinformatics to find the different protein structure, domains and chains and the amount of gene expression in different tissues and how splicing in various populations. Results: Different genotypes affect the penetrance and resistance and intensity and it can detect the population reaction to the various methods of diabetic cures. Conclusions: the result of this research can help us to predict the epidemic and decrease or increase of disease frequency in various populations and detect the harmful or useful migration and mate and heritability. This data can help us to get the better result of cure with the help of pharmacogenetic science.

p-434
IN SILICO INVESTIGATION OF THE INTRONIC REGIONS OF HUMAN VON WILLEBRAND GENE

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Von willebrand factor (vWF), a large glycoprotein encoded by a gene on the short arm of chromosome 12 that serves as a critical component in the blood coagulation pathway. The vWF gene spans 178 kp and consists of 52 exons ranging in size from 40 to 1,379 bp and its intron sizes varies between 97 bp to nearly 19.9 kb. With the aim of detection and characterization of possible regulatory elements in vWF introns, its intronic sequences was compared to the parts of human factor IX intron-1 sequences whose regulatory functions had been suggested previously. In the present study the possible presence of such elements and their distribution pattern within the vWF gene with 51 introns was investigated. More than 40% of the intronic sequences of vWF are composed of repeated elements. The present comparative study has resulted in detection of several repeated sequences with different sizes within intronic regions of vWF, with more than 75% homology to some of repeats in the intronic regions of hFIX and hFVIII genes. Similar to the pattern in hFVIII

intronic regions, reported in parallel, a non-random distribution pattern of repeated sequences is observed among vWF introns. The detected elements belong to sub-families of retrotransposon, known as Alu sequences. Analysis of such repeated elements has detected several motifs with transcription-factor binding activity that supports the possible presence of gene expression regulatory elements within the vWF intronic regions.

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CHARACTERIZATION OF A NOVEL CHITINASE FROM B. PUMILUS (CHIS) USING BIOINFORMATICS METHODS

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Protein evolution is new technology in molecular biology and biotechnology science which lead scientist to manipulate enzyme /protein to make proteins with high activity and function. Before any changing proteins in lab, theoretical methods using bioinformatics tools can help to predict and analyze proteins. Furthermore, bioinformatics can show pathway of workbench researches. Chitinases are a huge family enzymes that can degrade chitin polymer into the soluble N-acetyl D- glucosamine. Chitinase are produced by different microorganisms which generally present a wide multiplicity of enzymes. The perspective of chitinases in wide range of biotechnological applications is so positive particularly in the production of chito-oligosaccharides and N-acetyl D-glucosamine, biocontrol of pathogenic fungi, preparation of sphaeroplast and protoplast from yeast and fungi species also as natural pesticide and bioconversion of chitin waste to single cell protein. Novel chitinases from B. pumilus which previously isolated from salty soils of Gavkhooni marsh, Iran, have Salt-tolerance property. Two Chitinases named in the base of their length, Large Chitinase (ChiL), 696 amino acids with molecular weight of approx. 77 KDa and Small/Short Chitinase (ChiS), 596 amino acids with molecular weight of approx. 63 KDa. Analysis of phylogenetic tree shows that ChiL and ChiS are two different subfamilies: ChiS categorized in the subfamily of gram positive while ChiL is close to gram negative subfamilies. On the other hand, phylogenetic tree predicted that salt-tolerance activity of other subfamily of ChiS such as DAU101. Furthermore Global propensity of ChiS is indicated that Glu and Asp amino acids are relatively high which is not unusual in most halophil proteins but the propensity of Lys as positive charge (1.33) and the sum of hydrophobic residues particularly in C-terminal of enzymes is significant which tend to categorized this chitinase differently in family 18 glycoside hydrolases.

Cancer

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CAMPARISON OF THE EFFECTS OF ARSENIC TRIOXIDE AND ORGANO ARSENICAL COMPOUNDS, DIMETHYLARSINIC ACID & 1-NAPHTHYLARSONIC ACID, ON IN VITRO MICROTUBULE POLYMERIZATION

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Microtubules are dynamic polymers that have important roles in eukaryotic cellular processes such as signal transduction, cell polarity, and vesicular and chromosomal movements. Different compounds have been used as anticancer agents to inhibit microtubule polymerization .Because of the acute toxicity of such compounds; we searched for effective chemicals with low toxicity. We studied the interaction of arsenic trioxide, dimethylarsinic acid (DMA) and 1-naphthylarsonic acid (1-NAA) on microtubule polymerization under in vitro conditions. Tubulin was extracted from sheep brain and the experiments were conducted by adding appropriate amounts of GTP to tubulin preparations in PEM buffer at 37 ^{o} C. Three phases of nucleation, elongation and steady state was observed at 350nm spectrophotometrically. The results showed increasing lag time or nucleation step by 1.5 and 2 fold for 1-NAA and DMA respectively. Therefore it seems that these compounds inhibit microtubule polymerization via their effect on nucleation step. However arsenic trioxide affected lag time and elongation of tubulin polymers. Electron microscopy showed microtubular length decrement due to interaction of arsenic trioxide. We suggest that arsenic trioxide inhibits tubular polymerization by interaction with Mg^{2+}, which results in enhancing depolymerization, but organoarsenical compounds inhibit polymerization from the first step.

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EVALUATION OF PROSTATE SPECIFIC ANTIGEN (PSA) AND SIALIC ACID LEVELS IN PROSTATE CANCER

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This study aimed to measure the levels of sialic acid and prostate specific antigen (PSA) in prostate cancer patients and to compare the values with healthy control subjects., Patients and methods: Twenty seven patients having prostatic cancer were included in this study and were compared with thirty two healthy individual for both total sialic acid (TSA), lipid associated sialic acid (LSA), bound sialic acid (BSA) and PSA.. Results: There were significant increases in TSA ($P<0.001$), LSA ($P<0.01$), BSA ($P<0.001$) and PSA ($P<0.01$). Specificities and sensitivities were as follows: PSA (88% and 80 %), TSA (80% and 75%), BSA (60% and 63%), LSA (69%

and 70%), and FSA (65% and64%), respectively. Conclusion: The data obtained in our study suggest that the difference between the prostate cancer and control groups is reflected more significantly by PSA and sialic acid lacks tumor specificity and sensitivity and could not be used in screening tests for prostate cancer.

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THE NEW THEORY OF CARCINOGENESISTHE THEORY OF GENE MULTIPLE HITS

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Objective: In order to find the cancer development mechanism, including gynecological cancer, for the best cancer cure and treatment. There is no report about the new theory of gene multiple hits. Which is identified through a MEDLINE search of the English-language literature on "The New Theory of Carcinogenesis.The Theory of Gene Multiple Hits" and (or) the key words of this paper. 2. Method: summarizes the cancer scientific research findings. All the explanations for the new theory can be supported and understood by the research findings in cancer related statistics; cancer molecular statistics and cancer molecular biological researches. These findings should not be mentioned one by one. 3. The result of new discovery: explanation of the gene multiple hits theory we have known that cancer development is caused by the long time carcinogens' effects. The carcinogens include environmental or chemical factors; biological factors; physical factors and hereditary factors etc. Which all these factors display complicated effects on human body. At the final stage, the cancer is developed. All these complicated carcinogens, through many different ways, finally damage many different genes on the chromosomes. Which leads to develop cancer. The present molecular biological researches indicate that the cancer development involves two groups' genes which contain two different principle ways. One way is that many different carcinogens, through different ways, damage and affect the same or (and) different proto-oncogenes. Which makes them as oncogenes.The oncogenes, through different ways, promote or enhance the cell proliferation and finally cause carcinogenesis. At the same, the other principle way is that many different carcinogens, through different ways, damage and affect the same or (and) different tumor suppressive genes or anti-oncogenes. This makes the tumor suppressive genes dysfunction or losing their functions. The dysfunctioned tumor suppressive genes, through different ways, promote or enhance the cell proliferation and finally cause carcinogenesis. After all, the author may say in this way that before the cancer development, there are many different proto-oncogenes and tumor suppressive genes suffer from many different carcinogens' hits and damages. And finally the cancer is developed. 4. Conclusion: the significances of the gene multiple hits theory the significances of the new theory are great. The new theory not only fully explains the research findings for cancer development mechanism. But also summarizes the huge lots of different scientific research findings and using the single new theory represents the whole related scientific research findings. The new theory clearly addresses the cancer development mechanism. Which indicates the new theory is a very good theory. The gene multiple hits new theory provides the best directive references for the further cancer research, prevention, early diagnosis, early cure and cancer treatment.

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INDUCTION OF APOPTOSIS IN K562 CELL LINE AND DOUBLE STRAND BREAKS IN COLON CANCER CELL LINE EXPRESSING HIGH AFFINITY RECEPTOR FOR GRANULOCYTE MACROPHAGE-COLONY STIMULATING FACTOR (GM-CSF)

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Background: Immunotoxins are composed of both the cell targeting and the cell killing moieties. We previously established a new immunotoxin, i.e. StxA1-GM-CSF, composed of the catalytic domain of Shiga-toxin, as a killing moiety and GM-CSF, as a cell-targeting moiety. In this study the ability of this immunotoxin to induce apoptosis and double strand breaks in different cell lines was investigated. Methods: The recombinant hybrid protein was expressed in a bacterial expression system and purified with nickel-nitrilotriacetate acid resin. The K562 (Erythroid leukemia) cell line and LS174 (Colon carcinoma) were used in this study. The neutral comet assay was carried out for the detection of double strand breaks (DSBs) and for apoptosis, Hoechst staining was performed. Results: StxA-GM-CSF effectively induced apoptosis in K562 cell line and double strand DNA breaks (DSBs) were observed in colon cancer cell line treated with StxA1-GM-CSF. Conclusion: StxA-GM-CSF effectively induces apoptosis in K562 cell line. A novel phenomenon i.e. double strand DNA breaks (DSBs) was observed in colon cancer cell line treated with StxA1-GM-CSF. This novel action i.e. DNA damage might be a relevant mechanism of action for StxA1-GM-CSF which is designed to act as an immunotoxin, although further investigation is required.

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EFFECTS OF SILIBININ ON THE METASTASIS OF HUMAN PROSTATE ADENOCARCINOMA (PC-3) CELL LINE

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Prostate cancer (PCA) is the most common cancer diagnosed in men and the second cause of death due to cancers after lung cancer. Metastasis of cancer cells involves multiple processes and various cytophysiological changes, including adhesion capability changes among cells and the extracellular matrix (ECM) and damaged interacellular interactions. Silibinin, a naturally occurring flavonoid antioxidant found in the milk thistle, has recently been shown to have potent anti proliferative effect against various malignant cell lines, but underlying mechanism of action remains to be elucidated. In the present study, the PC-3 cells were incubated with different concentrations of silibinin for different times, then, cell cytotoxicity, cell adhesion and cell motility were assessed using MTT assay, cell-matrix adhesion assay and cell migration assay, respectively. The results showed that silibinin exerted dose- and time dependent inhibitory effects on the viability, motility and adhesion of highly metastatic PC-3 cells. These observations indicated that silibinin inhibits metastasis in a prostate cancer cell line.

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EXPRESSION OF NEUTROPHIL GELATINASE - ASSOCIATED LIPOCALIN (NGAL; LIPOCALIN 2, LCN2) IN DIFFERENT CANCEL CELL LINES

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The lipocalin superfamily, which includes the mouse and human homologues, 24p3/lcn2 and neutrophil gelatinase-associated lipocalin (NGAL), comprises a group of small extracellular proteins with a common β-sheet–dominated 3-dimensional structure. The pathophysiologic functions of 24p3/lcn2/NGAL are unclear, but it has been suggested that they may act in an immunomodulatory role by binding or inactivation of bacterial products, or through direct actions on the inflammatory cells. The expression of NGAL in several cancers caused it to be considered as an oncogene. However, the ocogenic role of NGAL/Lcn2 is controversial. In this study expression of NGAL/ Lcn2 in different cancer cell lines compared to normal cell lines was investigated. Human cancer cell lines, HepG2 (human hepatoma), K562 (erythroid leukemia), HL60 (acute myelogenous leukemia), U937 (monocytic leukaemia), A549 (breast carcinoma) and LS174T (Colon carcinoma) and two normal cell lines, fibroblast and mesanchymal stem cells were grown in RPMI-1640 medium with 10% fetal bovine serum . Total RNA was extracted and cDNA was synthesized. The assessment of NGAL/Lcn2 expression was performed by real- time RT-PCR and ELISA. A549, LS174T and HepG2 cell lines expressed more NGAL/Lcn2 compare to other cancer cell lines and normal cells. Up regulation of NGAL/Lcn2 in hematopoietic cancer cell lines, U937, HL60 and K562, was not observed. Since it was not observed in all cancer cell lines studied in the present study, it seems that up-regulation of NGAL/LCN2 depends on the type of cancer. Further studies are still required to address the reason/ reasons for NGAL/Lcn2 induction in cancer.

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STUDY OF PROSTATE SPECIFIC ANTIGEN GENE EXPRESSION AND TELOMERASE ACTIVITY IN BREAST CANCER PATIENTS

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Background and objectives: Breast cancer is the most common disease in women. Molecular tumor markers including prostate specific antigen and telomerase are engaged in this type of carcinoma. The aim of this study was to evaluate relationship between telomerase activity and prostate specific antigen gene expression in breast cancer patients and controls. Materials and methods: This was a case-control study and consisted of 25 women diagnosed with benign breast tumors as control and 35 women with malignant tumors as the cases. Telomerase activity was measured in tumor cytosols by TRAP assay. PSA protein was measured using ultra sensitive immunofluorometric assay. PSA mRNA expression was determined using RT-PCR techniques in all tumor tissues. Results: Using TRAP assay, the presence of telomerase activity was observed in all of the breast cancer patients. The relative telomerase activity (RTA) difference between the stages and the grades of breast tumors were statistically significant ($p<0.05$). PSA mRNA were detected only in benign tumors and particularly in stage I and grade I malignant tumors. PSA levels between the cases and control groups and also among all grades and stages of the disease were significant ($p <0.05$). There was an inverse significant correlation between the RTA and PSA protein levels in the case groups ($r=-0.42$, $p<0.05$). Conclusion: Our results showed that there was a reverse relationship between PSA mRNA expression and increased telomerase gene expression during breast cancer progression and development. Measurement of telomerase activity could be a favorable biomarker along with PSA in breast cancer diagnosis.

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PROSTATE SPECIFIC ANTIGEN GENE EXPRESSION AND TELOMERASE ACTIVITY IN BREAST CANCER PATIENTS: RELATIONSHIP TO STEROID HORMONE RECEPTORS

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Background and objectives: Breast cancer is the most common disease in women and is usually a steroid hormone receptor dependent cancer. The aim of this study was to evaluate the relationship between telomerase activity and prostate specific antigen gene expression with steroid hormone receptors in breast cancer patients. Materials and methods: This study consisted of 50 women with benign breast tumors and 50 malignant tumors. Telomerase activity was measured in tumor cytosol samples by telomeric repeat amplification protocol (TRAP) assay. PSA protein and mRNA expression were carried out using ultra sensitive immunoassay and RT-PCR techniques in all tumor tissues, respectively. Estrogen and progesterone receptors were stained using immunohistochemistry in tumor tissues. Results: Presence of the telomerase activity was positive in all of the breast cancer patients. The difference of relative telomerase activity (RTA) values between stages and grades were more statistically significant ($p<0.05$). The PSA mRNA was detected only in benign tumors and stage I and grade I malignant tumor cytosols. Difference of tumor cytosol PSA levels between the cases and control groups and also among all grades and stages of the disease were significant ($p <0.05$). There was an inverse significant correlation between the RTA and PSA protein levels in the case groups. ($r=-0.42$, $p<0.05$).There was a statistically significant difference between ER and PR positive and PSA negative and telomerase activity ($p<0.05$) Conclusion: It is speculated that differential expression of PSA and telomerase genes in breast tumors are under control of steroid hormone receptors and could be used as a target for treatment.

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EFFECT OF ZINC, SELENIUM AND COPPER ON TELOMERASE GENE EXPRESSION IN BREAST CANCER CELL LINES

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Background and objectives: Breast cancer is the most common cancer in women. The aim of this study was to evaluate the effect of zinc, selenium and copper on telomerase gene expression in a breast cancer cell line. Materials and Methods: T47D breast cancer cell line was cultured and treated with different concentrations of trace elements (Zn, Cu, Se). The kinetic study was carried out on telomerase gene expression. Relative telomerase activity (RTA) of the cell line (T47D) was measured by TRAP assay method. Results: After treatment with 100 μM $ZnSO_4$, telomerase activity of T47D cells was markedly increased after 6 hr (5.2 fold). Treatment with 100 μM $ZnSO_4$ for 24 hr and 500 μM $ZnSO_4$ for 6, and 24 hr resulted in telomerase activities of 0.76, 0.39 and 0.12%, respectively (control 49.2%). Treatment with 10 μM $CuSO_4$ increased the telomerase activity of T47D cells markedly after 6 hr (3.67 fold). However, there was no significant effect at a concentration of 3 μM $CuSO_4$ after 6 hr. The concentrations of 3 and 10 μM $CuSO_4$ had no effect at 24 hr. Treatment with 10 and 30 μM selenium-l- methionine inhibited telomerase activity of T47D cells markedly. Telomerase activities of T47D cells were 0.93, and 0.60% after 24hr and 0.76, and 0.12% after 48 hr, respectively (control 49.2%). Conclusion: It is speculated that changes of trace elements particularity Se, Zn and Cu may have inhibitory effects on breast cancer cell lines and could be a target for therapeutic purposes.

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THE EFFECTS OF ANTINEOPLASTIC AGENT MITOXANTRONE ON CHROMATIN COMPONENTS OF LIVER NUCLEI

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Mitoxantrone is an antineoplastic agent, widely used in the treatment of various cancers such as advanced breast cancer and lymphoma. It binds to DNA and induces single and double strand breaks and strongly inhibits DNA and RNA synthesis .In the present study, we have investigated the binding of mitoxantrone to soluble chromatin and DNA. Nuclei were prepared from rat liver and after brief digestion with micrococcal nuclease; the soluble chromatin was treated with different concentrations of mitoxantrone. Also in comparison, DNA was isolated from chromatin and after treatment with mitoxantrone; it was analyzed in the same condition used for the chromatin. To monitor the amount of turbidity, absorbance at 400 nm was measured. The results show that in the case of chromatin the highest degree of turbidity is achieved at 40 μ M. Whereas in the case of DNA, turbidity s occurred at higher concentration of the drug (80 μM)Also the protein and DNA contents were analyzed on SDS and agarose gels, respectively. The results show that the amount of histones on SDS gel is diminished as the drug concentration is increased and at 40 μM of drug, a faint or no histone band was visible on the gel, compared to the control. The result of the electrophoresis of DNA treated with mitoxantrone show that the content of DNA is decreased as drug concentration is increased, confirming the turbidity results .In conclusion it is suggested that the mitoxantrone has higher affinity to chromatin than to DNA and the interaction is accompanied by chromatin aggregation.

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COMPARISON OF ADRIAMYCIN AND IDARUBICIN EFFECTS ON RELEASING NUCLEAR PROTEINS FROM NUCLEI

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In the present study, the effect of anticancer drugs, adriamycin and idarubicin (4-methoxy daunomycin) on chromosomal proteins and the possible release of these proteins from the chromatin have been investigated. The nuclei were isolated from rat liver and exposed to various concentrations of adriamycin or idarubicin for 45 min at room temperature in the dark. The samples were centrifuged and the supernatants were analyzed using SDS-polyacrylamide gel electrophoresis and UV/Vis spectroscopy techniques. The results show that gradual increase in drugs concentration reduced the proteins released into supernatant. SDS-polyacrylamide gel electrophoresis showed higher affinity of adriamycin to chromatin compared to idarubicin. Thus at low concentration of adriamycin, a considerable decrease in the histones content was observed whereas in the case of idarubicin, higher concentrations of the drug was needed to obtain the same result. Absorbance changes at 210 and 480 nm also confirmed the results. Interestingly when the concentration of adriamycin was increased to 200 µg/ml (drug/DNA ratio 2:1) a new protein band with a molecular weight of 12 kDa appeared in the position of HMG N1. The results suggest that although adriamycin and idarubicin exhibit a common structural feature, they differ in their effect on nuclei and chromatin, suggesting that idarubicin represents lower toxicity than adriamycin, at the chromatin level.

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CORE HISTONE PROTEINS AS A TARGET OF AN ANTHRACYCLINE ANTIBIOTIC, DAUNOMYCIN, STUDIED IN SOLUTION

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Daunomycin is an anthracycline anticancer drug widely used in cancer chemotherapy. The binding of drug to DNA and chromatin has been extensively studied but the interaction of this drug with the core histone is still unknown. In the present study, we have investigated the binding of daunomycin to thymus core histones free in solution or cross-linked with bifunctional reagents. The results demonstrate that fluorescence emission intensity is decreased as drug concentration is increased but drug shows higher affinity to free histone rather than to the cross-linked one. The results also confirmed using UV /Vis spectroscopy. Equilibrium dialysis experiments show a cooperative binding pattern and reveals that daunomycin binds with higher affinity to free histones. It is suggested that daunomycin binds to open state of chromatin, the condition that is usually found in transcriptionally active chromatin and in tumor cells.

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DIAGNOSIS OF PROSTATE CANCER IN URINE BY GSTP1 HYPERMETHYLATION DETECTION

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Background: Prostate cancer is the most commonly detected male cancer and the second leading cause of male cancer deaths in the United States. New techniques for the early detection and management of prostate cancer are needed. The study of genetic alterations has been used as targets for the detection of neoplastic cells in body fluids like urine. Hypermethylation of normally unmethylated CpG islands in the promoter regions of tumor suppressor genes correlates with loss of gene expression in human tumors. Hypermethylation of regulatory sequences at the detoxifying GSTP1 gene locus (Glutathione S-transferase P1) is found in the majority of primary prostate carcinomas but not in normal prostatic tissue or other normal tissues, nor in benign prostate hyperplasia (BPH). Objectives: It is important to understand the molecular mechanisms of prostate cancer initiation to design cancer-specific diagnostic techniques during early stages of the disease for diagnosis and management of the cancer. So, the goal of this study is to consider the potential role of this epigenetic alteration as a biomarker for early detection and develop specific and non-invasive tests for diagnosis and monitoring of prostate cancer. Materials and methods: Matched specimens of primary tumor, peripheral blood lymphocytes (normal control) and urine were collected from patients with prostate cancer. GSTP1 hypermethylation was detected by sensitive method of methylation specific-PCR (MS-PCR). Genomic DNA was isolated from the samples and modified by sodium bisulfite. Then, PCR was performed with GSTP1 primers specific for the methylated and unmethylated regions, separately. Results: The results showed that 79% prostate of tumors were positive for GSTP1 methylation. In 27% of the cases, the corresponding urine-sediment DNA was positive for GSTP1 methylation, indicating the presence of neoplastic DNA in the urine. Furthermore, there was no case in which urine-sediment DNA was methylated when the corresponding tumor was negative. So, molecular diagnosis of peostate cancer in urine is feasible. Conclusion: This finding suggests that GSTP1 promoter methylation study can serve as a new molecular diagnostic tool to aid in prostate cancer management and detection using body fluids like urine sediments. Also, because methylation alteration is reversible, it may lead to the useful application of demethylating drugs for the treatment of prostate cancer in future.

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EXPRESSION OF ADENOSINE RECEPTORS IN THE HUMAN LUNG ADENOCARCINOMA CELL LINE (CALU-6)

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The local release of adenosine in hypoxic tumors may regulate the growth and development of the tumor cells in adenosine

receptor-dependent and independent ways. Consequently, the presence of defined receptor subtypes will be an important determinant for a specific effect of adenosine on the function of a tumor cell, which may aid in the development of potential therapeutic targets. Recently we have identified the expression profile, signal transduction, molecular function and cell growth modulation of adenosine receptor subtypes in the human breast and prostate cancer cell lines. To investigate the possible roles of adenosine receptors in other types of human cancers, in this study, we characterized the expression profile of adenosine receptors in the human lung carcinoma cell line (Calu-6). Our purpose is to test the hypothesis that diverse human cancer cell lines, according to their adenosine receptor subclass status, would show differential growth modulation and that this will help tumor growth inhibition. RNA was extracted and reverse transcribed to cDNA. PCR primers were synthesized from human adenosine receptor cDNA sequences. PCR was performed under optimized conditions for each receptor subtype. Amplification of β-actin mRNA served as control for RT-PCR. The PCR products were separated on 1.7% agarose gels. Preliminary RT-PCR results revealed expression of the adenosine A1, A2A and A2B receptors but no expression for A3 subtypes in the human lung carcinoma cell line. Further work(s) will be required to understand the functional roles of expressed adenosine receptors in the human lung cancer cell growth and development.

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MOLECULAR ANALYSIS OF P53 AND MEN1 EXPRESSION IN MCF7, T47D AND MDA-MB 468 BREAST CANCER CELL LINES TREATED WITH ADRIAMYCIN USING RT-PCR AND IMMUNOCYTOCHEMISTRY

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Breast cancer is one of the leading causes of mortality among women worldwide. The p53 is a well-known tumor suppressor gene commonly mutated in malignancies including breast cancer. It is involved in the regulation of cell cycle, DNA repair and apoptosis. MEN1, another tumor suppressor gene, encodes a nuclear protein called menin with a variety of functions including regulation of transcription, cell proliferation and apoptosis. In addition, estrogen receptor (ER), an important prognostic factor, is expressed differentially in breast cancer cells. Therefore, we decided to study the p53 and MEN1 expression at both mRNA and protein levels using RT-PCR and immunocytochemistry in MCF7, T47D and MDA-MB-468 breast cancer cell lines with different ER status following exposure to Adriamycin (ADR). The cytotoxicity of ADR on tested cell lines was also determined using MTT method. ADR showed different dose and time-dependent anti-proliferative effects in these cell lines. The p53 mRNA level didn't change significantly after ADR treatment in these cell lines while in MDA-MB 468 and MCF7 cells, MEN1 mRNA showed a slight decrease after ADR exposure. MEN1 mRNA level was highest in MDA-MB 468 and lowest in MCF7 cells. Higher levels of menin and p53 protein expression were detected after ADR treatment in all tested cell lines. In conclusion, these cells showed differential molecular responses to Adriamycin which is important in tumor-targeted cancer therapy. Meanwhile, these data emphasize the requirement of new biomarkers such as menin to be used in combination with current markers for prediction of response to chemotherapy.

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HEREDITARY BREAST CANCER DNA BANK IN IRAN: NECESSITY TO DETECT FAMILY MEMBERS SUSCEPTIBLE TO BREAST CANCER

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DNA bank refers to a facility that stores DNA for future analysis. It enables us to share samples and repeat experiments. Iranian Center for Breast Cancer in corporation with Iranian Molecular Medicine Network established a DNA bank for hereditary breast cancer to facilitate future research in the field of breast cancer. In our familial cancer clinic, through genetic counseling we gather required clinical data, draw pedigree by Progeny Software Ver5.5 and estimate the risk of breast cancer for each individual. After assignment of informed consent, we collect blood samples from patients and their first and second-degree relatives and extract DNA in our genetic laboratory. In a period of one year, we collected more than 100 DNA samples from 19 families suspected of hereditary breast cancer. The mean total number of breast cancer cases in these families was 2.6 and in 3 families we observed both ovarian and breast cancers. In one family there was a history of bilateral breast cancer. Hereditary breast cancer accounts for about 5-10% of all breast cancers and occurs as a result of highly penetrant mutations in solitary genes. Some of these genes have been identified t but most of them were still unknown. DNA bank would provide enough DNA samples for research regarding the genetic aspects of breast cancer. Lack of immediate benefits for patients and poor knowledge of patients and even physicians are of great obstacles in DNA banking.

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OXIDATIVE STRESS AND ANTIOXIDANT STATUS IN CANCER PATIENTS

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Background and objectives: Imbalance of oxidant –antioxidant systems plays an important role in the incidence of cancer. The present study was carried out to determine, oxidative stress and total serum antioxidants (TAS) in cancer patients. Besides, serum copper levels in cancer patients were also stud SO4ied because it may have a promoting role in the formation of MDA. Material and Methods: Malondialdehyde (MDA), total antioxidant status, and serum copper levels of 49 cancer patients aged 19-80 years and 18 healthy subjects (control group) aged 22-76 years were evaluated. Serum concentrations of MDA as thiobarbituric acid reactive substances, serum TAS and copper levels were measured using a fluorometric method, a commercial kit from Randox Laboratories and flame atomic absorption spectrophotometry, respectively. Patients were classified on the basis of the location of tumors as esophagus, head and neck, colorectal and lung cancer groups. Data were analyzed by descriptive statistics, one-way Anova and correlation between copper and MDA by Sperman test. Results: The mean serum MDA concentrations of all cancer groups except lung cancer group were significantly higher than the control group ($P<0.004$). The mean total antioxidant statuses of all groups were higher than control group but the difference was not significant. The mean serum copper of all cancer groups except colorectal cancer group was significantly higher than the healthy subjects ($P<0.05$). We did not observe a significant correlation between copper and MDA levels. Conclusion: An alteration in the lipid peroxidation with concomitant changes in antioxidant defense system in cancer patients may be due to excessive oxidative stress. In this study there was no significant correlation between serum copper and serum MDA levels, which may be due to small sample size. The increased serum copper levels in our patients may indicate active cancer.

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A MICROARRAY APPROACH TO INVESTIGATE GENOMIC IMPACTS OF CATIONIC LIPID-BASED GENE DELIVERY NANOSYSTEMS IN HUMAN ALVEOLAR EPITHELIAL A549 CELLS

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Viral and non-viral vectors are widely exploited for gene therapy in vitro and in vivo. However, their genomic impacts in target cells/tissues are yet to be fully understood. In the current investigation, we studied the cyto- and geno-compatibility of cationic lipid, oligofectamine (OF) and its lipoplexes with DNA (OF: DNA), in human alveolar epithelial A549 cells recruiting DNA microarray technology followed by cytotoxicity examinations (e.g., flow cytometry and comet assay). The preliminary cytotoxicity examinations revealed inevitable apoptotic effects within A549 cells upon treatment with OF or OF: DNA nanostructures. For microarray-based geno-compatibility analysis, RNA samples from treated and untreated cells were converted to aminoallyl-cDNA, labeled with cyanine (Cy3 or Cy5) and hybridized on target arrays housing 200 gene spots. Data were subjected to the Lowess normalization and revalidation with RT-PCR analysis. Microarray examinations resulted in marked changes (≥2-fold) in gene expression belonging to the various genomic ontologies such as cell defense and apoptosis pathways, namely as follows: OF-upregulated: IL9R, TNFRSF6, and PSMA4; OF-downregulated: CDK4, TNFRSF6, SEP2, and PSMA4; and OF: DNA-upregulated: CD14, and CTSG; and OF:DNA-downregulated: TNFRSF6, CXCR4, and POU2AF1. The microarray results were revalidated by RT-PCR analysis. Although flow cytometry examinations revealed apoptosis within the treated cells with nanostructures, however comet assay showed no DNA damage. Upon our findings on the genomic impacts of cationic lipids, it is suggested that undesired intrinsic transcriptomic alterations by cationic lipid gene delivery nanosystems should be taken into account when they are used as gene delivery systems.

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COMPARISON OF THE DRUG RESISTANCE OF GASTRIC CANCER CELL LINES BY HUMAN CALPROTECTIN AND ANTICANCER DRUGS

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Calprotectin and etoposide are cytotoxic agents with anti-tumor effects through suppression of topoisomerase II; a key enzyme in apoptosis. In the present study, cytotoxic effects of calprotectin and anticancer drugs etoposide, cisplatin and doxorubicin acting similar to cyclosporine in inhibiting the expression of MDR and penicillin that enhances the expression of MDR were investigated in AGS cell line. AGS cells were exposed for 48 hr to the LC50 concentrations of both calprotectin and the anticancer drugs associated to cyclosporine and penicillin. Cells exposed to none of these drugs were used as negative controls. Cell proliferation was assessed using MTT assay. Our results revealed that combination of both calprotectin and anticancer drugs associated with cyclosporine induce growth inhibition more than the toxic agents used alone, while a combination of calprotectin with penicillin showed opposite results. Since anticancer drugs are lipophilic and calprotectin has cell surface receptors and both demonstrate similar effects on MDR expression, further investigation on the molecular mechanism of such drugs are required.

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THE SIGNIFICANCE OF LDL AND HDL SUBCLASSES IN THE ASSESSMENT AND MANAGEMENT OF CHD RISK

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Coronary Artery Disease (CAD) is the leading cause of death in the world. Total Cholesterol is recognized as the main risk factor for CAD. Also, it has been shown that HDL-Cholesterol

has a negative and LDL-Cholesterol a positive correlation with CAD. It has been established that individuals with "normal" HDL and LDL Cholesterol levels will develop CAD and conversely, so many individuals with "adverse" HDL and LDL levels never develope the disease. Yet recently, a growing body of evidence suggests that measurement of lipoprotein subclass particles in plasma is a more accurate predictor of CAD. These particles are heterogenic with respect to size, density, and chemical composition. Small and dense LDL particles are more atherogenic than other LDL particles. Small and dense LDL particles are readily oxidized and enter the arteries. Furthermore, these particles are responsible for delayed clearance of atherogenic remnant particles. Two phenotypes have been described. Phenotype A is associated with larger, buoyant LDL particles, whereas phenoytpe B is associated with a predominance of small and dense LDL particles. In a study by Austin MA et al. the presence of a dense LDL phenotype was proposed as a genetic marker for risk of Coronary Heart Disease. Currently, there are 5 or 6 methods available for measuring HDL, LDL, and VLDL sub-fractions, which will be discussed briefly. As a result of these findings, it is crucial for physicians to order a more comprehensive lipid panel containing both routine and advanced HDL and LDL sub-frationation testing.

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DIFFERENTIAL EXPRESSION OF SURVIVIN & ITS SPLICE VARIANTS; 2B & ΔEX3 IN THYROID NODULES: PROMISING NEW MOLECULAR MARKERS IN PAPILLARY THYROID CARCINOMA

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Survivin is a new member of the protein family of apoptosis inhibitors that has been recently attracted scientists' attention as a molecular marker in cancer research. Recent studies indicate the differential expression of survivin and its splice variants in cancer versus normal adult cells. Because of the heterogeneous nature of tumor and non tumor thyroid nodules as well as the lack of a suitable molecular marker for diagnosis and prognosis of papillary thyroid carcinoma, the aim of this study was to evaluate the potential usefulness of survivin and its splice variants; 2b & ΔEx3 as new molecular markers in papillary thyroid carcinoma. Tissue cases were collected from 61 thyroid specimens including normal samples (14), non-tumor nodules (11), benign tumors (11) and malignant ones (25). mRNA levels were measured by semi quantitative RT-PCR and normalized by ß2m as an internal control. Our result showed that: 1) survivin and survivin ΔEx3 are differentially expressed in tumors rather than the normal and non-tumor tissues. 2) The transcription level of survivin 2b was significantly lower in tumors compared to non-tumor ones. 3) The expression level of survivin and survivin ΔEx3 was significantly ($P<0.05$) correlated with malignant nature of tumors. In conclusion our data revealed, for the first time, that the expression of survivin and survivin ΔEx3 is limited to tumor tissues and their expression levels are associated with high stages III & IV of papillary thyroid carcinomas. Therefore, evaluating survivin gene expression and its splice variants might have a potential usefulness in diagnosis and classification of thyroid tumors from non-tumoral nodules as well as prognosis of papillary thyroid tumors.

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CTLA-4 GENE VARIANTS IN PATIENTS WITH LUNG CANCER

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Background: Ten millions of people are diagnosed with lung cancer each year. There is no validated screening method for lung cancer even in high-risk individuals. Objectives: To investigate the possible association of ctla-4 gene variants with lung cancer and its comparison with normal subjects. Methods: ctla-4 gene polymorphisms were investigated in 124 lung cancer patients and 122 age and sex matched healthy control subjects. Results: No significant association between genotype or allele frequencies of -318C/T and -1722 T/C SNPs in lung cancer was seen. There were no differences in genotypes/alleles frequencies between control group and non small cell lung cancer (NSCLC) alone. In addition, there was no statistically significant difference in CTLA-4 genotypes between small cell lung cancer (SCLC) and NSCLC. Conclusion: It appearss that allele and genotype frequencies of ctla-4 promoter are not associated with susceptibility to lung cancer.

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STUDY OF ERB-B2 EXPRESSION IN MALIGNANT BREAST TUMOR SAMPLES BY PCR IN COMPARISON WITH IMMUNOHISTOCHEMICAL TECHNIQUES

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Breast cancer is one the most common cancers among women and the fatality rate is relatively high. It seems that many mutations such as oncogene amplification and deletion or loss of function of tumor suppressor genes contribute to breast cancer development. Among oncogenes, erb-B2, which belongs to EGFR (epidermal growth factor receptor) family, is amplified in 25-30% of tumors with ductal cell origin. (Ductal cell carcinoma frequency is 80% of all breast cancers.) In the cases with erb-B2 amplification, the growth of tumors can be prevented to some extent using a humanized monoclonal antibody (Herceptin) against erb-B2 oncogene product (HER2/neu). Consequently, identification of tumors with erb-B2 amplification is important. The routine method to achieve this end is immunohistochemistry (IHC). In this study, in

order to compare RT-PCR with IHC techniques, fifty tumor sampels were collected from Dey Hospital. IHC techniques showed that 32% of these samples were erb-B2 positive. RNA extraction of the samples was done and an RT-PCR assay which simultaneously amplifies erb-B2 and beta globin as an internal control was performed. Following agarose gel electrophoresis, the intensities of erb-B2 band and beta globin were compared. It was demonstrated that RT-PCR efficiency for detecting amplification in tumor samples with +3 IHC grade is 100% and that of tumor samples with +2 IHC grade is approximately 100%. In addition, a PCR assay was performed on DNA extracted from both tumor samples and normal blood to confirm RT-PCR results.

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EVALUATING THE EXPRESSION OF SURVIVIN GENE AS A SPECIFIC TUMOR MARKER FOR PROGNOSIS OF BLADDER CANCER

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Inhibition of apoptosis may favor the onset and progression of cancer. Survivin is an inhibitor of apoptosis that have been considered as a novel diagnostic/prognostic marker of bladder cancer. The survivin protein regulates both cell division and cell survival and is over-expressed in the vast majority of human cancers. Recently, different splicing variants of this gene have been detected, which play various roles in controlling apoptosis. In this study, the expression level of survivin in bladder tumor tissues was assessed for the potential prognostic relevance. FFPE (formalin-fixed paraffin embedded) samples were collected from Labafi-Nejad Hospitsal and patients with a five-year history of bladder cancer were chosen for further analysis. Expression of survivin mRNA, was measured by hemi-nested RT-PCR analysis and tissue distribution and subcellular localization of survivin protein in tumor tissues was also examined by immuno-histochemistry (IHC). Our observations revealed a substantial expression level of survivin mRNA in tumor tissues of bladder. According to IHC results, survivin protein is expressed in cancer cells and is primarily localized in nucleus. In conclusion, we were able to detect expression of survivin mRNA and protein in FFPE bladder tissues.Further studies are in progress to examine the prognostic value of survivin expression in this disease in addition to the evaluation of the correlation between the level of expression of survivin and the clinicopathological characteristics of the tumors.

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MUTATIONAL ANALYSIS OF THE PTEN/MMAC1 GENE LOCALIZED AT CHROMOSOME 10Q23 IN ISFAHAN PATIENTS WITH SPORADIC BREAST CANCER

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Phosphatase and tensin homologue deleted on chromosome 10 (PTEN), a tumor suppressor gene located on chromosome 10q23, has recently been shown to act as a phosphatidyl inositol 3,4,5-triphosphate phosphatase and to modulate cell growth and apoptosis. Somatic mutations of this gene were detected in several malignancies including brain, prostate, and breast tumors. Previous genotype/phenotype correlations have identified several potential associations, for example, truncating mutations result in increased breast cancer risk. Considering the high incidence of breast cancer in Isfahan, the potential role of this gene in mammary carcinogenesis was further investigsted. We examined 62 metastatic breast cancers for mutations in PTEN/MMAC1 by means of polymerase chain reaction, single-strand conformation polymorphism and sequencing analysis. PTEN deletions/insertions and nucleotide changes were found in 4/62 (6%) and 9/62 (14%) of high-grade breast cancers, respectively. This observation is an important consideration for novel therapeutic trials such as the use of tetrocarcin, NSAIDs, and rapamycin in breast cancer chemotherapy in which biologic efficacy is influenced by the activity level of PTEN.

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STUDY OF ANTI-ANGIOGENIC ACTIVITY OF CAPPARIS SPINOSA FRUIT ON HUMAN BONE MARROW ENDOTHELIAL CELLS IN 3D FIBRIN GEL

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Angiogenesis, the formation of new blood capillaries from pre-existing capillaries and post-capillary venules, links with embryonic development and pathological conditions. Since angiogenesis is important in the pathogenesis of various diseases, the inhibition of angiogenesis, or anti-angiogenesis, is a promising approach in their treatment. In this study we extracted dried Capparis spinosa fruit meal in phosphate buffer overnight. Its anti-angiogenic activity was studied in a 3D microcarrier-based system. Different amounts of aqueous extract were added to human bone marrow endothelial cells in the presence of endothelial growth factor in 3D fibrin gel. After 3 days anti-angiogenic activity was studied in comparison with the control. In studied doses, aqueous extract of Capparis spinosa showed strong anti-angiogenic activity. The aqueous extract inhibits endothelial cell migration and tubulogenesis in fibrin gel. The results demonstrated its anti-tumor activity and confirmed its use in traditional medicine.

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ADIPOSE-TISSUE-DERIVED MESENCHYMAL STEM CELLS (MSC), AN ALTERNATIVE SOURCE FOR CANCER CELL THERAPY

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Mesenchymal stem cells (MSC) are known as self-renewable, multipotent cells which possess the capacity of differentiating into multi-lineage cells such as: chondrocytes, osteoblasts, adipocytes, neurons and hematopoietic-supporting stroma. MSCs have alternative sources in which adipose tissue is the main and ideal one, representing high quantities of adult stem cells. In this investigation, adipose tissue was isolated from breast cancer patients after radical mastectomy. The tissues were homogenized and the breast adipose cells were cultured in DMEM-HG media with 10% FBS. MSCs were isolated upon their attachment to tissue culture plates and subsequently sub-cultured for further proliferation and expansion. Flow cytometry analysis of cell surface markers of the isolated cells exhibited lack of expression of CD45, CD14, CD34, and a high level of expression (up to 90%) of CD166, CD105 and CD44, confirming the cells MSC nature. Unique characteristics of MSC including the retention of the potential for differentiating from one cell type to another, plasticity, easy harvest and the ability for gene transfection have made them effective for therapeutic purposes such as cell and gene therapy for the treatment of malignancies and degenerative diseases.

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SOMATIC MUTATION OF P53 ON CODON 179 IN IRANIAN WOMEN WITH BREAST CANCER

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Introduction: Breast cancer is a malignant tumor of breast tissue and the most prevalent cancer among women. The national cancer institute (NCI) estimates that about 1 in 50 women will develop breast cancer by age 50 and this ratio increases with age. Many risk factors such as race, genetic factors, family history, alcohol consumption, menstrual periods, menopausal periods, hormone replacement therapy (HRT) are involved. Screening tests are needed for diagnosis, prognosis and therapy of breast cancer. P53 is a tumor suppressor gene that its protein product acts as a regulator and a key element in DNA repair. Mutations in the P53 gene are the most frequent genetic changes in human breast cancer, and one of the most prevalent mutation sites of this gene is codon 179. Aim: Detection of P53 gene mutation in codon 179 in Iranian women with breast cancer by PCR-RFLP. Materials and methods: this study was performed by extracting DNA from primary breast cancer cells followed by the design of primers for the study of codon 179 with NTI-Vector software. Detection of codon 179 mutations was done by Nla III PCR_RFLP. Results: The somatic mutations of P53 on codon 179 were observed in 16% (7 of 53) of our cases; 14% with heterozygote and the rest with homozygote mutations. This ratio is the highest reported amount in the entire world. Conclusion: This study indicates that genotyping of P53 gene on codon 179 is important for Iranian women with risk factors for breast cancer .

p-464
EFFECT OF TUMOR NECROSIS FACTOR-ALPHA ON MATRIX METALLOPROTEINASE-2 ACTIVITY IN GASTRIC ADENOCARCINOMA AGS CELL LINE IN VITRO

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Matrix metalloproteinases (MMPs) have an essential role in angiogenesis, tumor invasion and metastasis by degradation of extracellular matrix. Cytokines produced in tumors, can increase MMPs production in cancer cells. A possible basis for gastric carcinoma has been implied to be persistent chronic inflammation. In this regard a role for pro-inflammatory cytokines has been suggested. In the present study, we investigated the effect of tumor necrosis factor-alpha (TNF-α) on MMP-2 activity produced by gastric adenocarcinoma AGS cell line. The AGS cells were cultured in complete RPMI medium. Then cells were incubated with different concentrations of human TNF-α (0.1 - 100 ng/ml) for 24, 48 and 72 hours. Afterward supernatants from AGS cell cultures were collected. Zymography was used to analyze the effect of TNF-α on gelatinolytic activity of MMP-2 in TNF-α-stimulated AGS cell line. TNF-α increased markedly the activity of MMP-2 in AGS cells dose-dependently from 24 hours onwards, compared with untreated control cells. Our results suggest that TNF-α substantially up-regulates MMP-2 activity in AGS cell line. So this cytokine can be involved in progression of gastric cancer by increasing the gelatinolytic activity of MMP-2, a collagenase with invasive and metastatic capacity, which leads to angiogenesis. Thus targeting of TNF-α could have potential implication for prevention of metastasis and consequent angiogenesis in gastric cancer.

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EVALUATION OF THE LEVEL OF THE NITRIC OXIDE PRODUCTION IN TWO CELL LINES OF HEPATOCELLULAR CARCINOMA (HEPG2) AND LARYNGEAL CARCINOMA (HEP2) IN THE PRESENCE OF WHOLE SAFFRON EXTRACT

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Background: A number of studies have demonstrated an antitumor effect of saffron and its constituents on different malignant cells in vitro. It has been reported that a novel glucoconjugate isolated from corms and callus of saffron possesses cytotoxic activity against different tumor cells with nitric oxide (NO) production. These data suggest that the cytotoxic effect of saffron extract is related to nitric oxide. Objective: To investigate and assess, nitric oxide (NO) production in hepatocellular carcinoma cell line (HepG-2) and laryngeal carcinoma cell line (Hep-2) in the presence of saffron extract. Design: Saffron extract was prepared. Cells were cultured, and treated with different doses of saffron extract (0, 200, 400 and 800 µg/dl). After 24, 48 and 72 hrs, respectively, the morphologic modifications were observed

and recorded. Moreover by using the MTT test, the quantitative changes were determined and assessment of the NO production was undertaken with the use of Griess test. Results: The morphologic images showed qualitative changes in two cell lines (HepG2 and Hep-2). The MTT test results indicated that there was an increase in cytotoxic effect with an increase in saffron extract concentration, however, the nitric oxide (NO) concentration, decreased significantly after 6, 12, 18, 24, 48 and 72 hrs respectively. Conclusion: This study suggests that the saffron extract has a cytotoxic effect on HepG-2 and Hep-2 cell lines and the cytotoxic effect is probably somehow related to a decrease in the nitric oxide (NO) concentration.

p-466
STUDY ON THE DEATH INDUCING ACTIVITY OF HUMAN CALPROTECTIN AND ETOPOSIDE (VP16) AGAINST AGS-CANCER CELLS

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Gastric cancer is still a leading cause of cancer related death in many parts of the world. This cancer alone is the cause of more than 750,000 annual deaths in the world and is the most common fatal cancer in Iran with a wide variation of death rate in different provinces. Previous studies revealed that etoposide induced apoptosis through Topo-II (Topoisomerase-II) inhibition. On the other hand one possible way for calprotectin apoptosis-inducing activity is through inhibition of casein kinases, preventing phosphorylation activation of Topo-II. In this study various concentrations of calprotectin, purified from human neutrophil, and etoposide, a standard cytotoxic drug, were incubated with AGS cancer cells for different times. Treated cells were seeded into 96-well plates (2×104cells/ml) and relative cell number was measured using 3-(4,5-dimythylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay and dye exclusion test. Our results demonstrate that incubation of AGS cells with calprotectin and etoposide lead to inhibition of AGS cell proliferation in a time and dose dependent manner. Maximum inhibition effect calprotectin and etoposide was observed at 70 and 30µg/m after 48 h incubation, respectively. This finding suggests an antagonizing effect between calprotectin and etoposide in a specified range of concentration which probably reflect a competition for specific binding sites on the plasma membrane or may be related to the ultimate common target molecule; Topo-II.

p-467
PROLIFERATION KINETICS, AN IMPORTANT FACTOR IN CELL GROWTH INHIBITORY EFFECTS OF CALPROTECTIN AND ETOPOSIDE

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Nowadays we know more than 100 kinds of different tumors; each with a specific response to anti-tumor agents. The response depends on specific characters of the tumor e.g. size, location, rate of differentiation and some biological factors. In this study 12 different cancer cells from different origins were cultured with two anti-tumor agents, calprotectin (purified from human neutrophil with an inhibitory proliferation effect) and etoposide (a standard chemotherapeutic agent) for 48h. Treated cells were seeded into 96-well plates (1×104cells/ml) and relative cell number was measured using 3-(4,5-dimythylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay. Our result suggests that growth inhibitory effect of both cytotoxic agents depends on the proliferation kinetics of the tumor cells.

p-468
COMPARATIVE GENOMIC HYBRIDIZATION ANALYSIS (CGH) OF SQUAMOUS CELL CARCINOMA OF ESOPHAGUS IN AN IRANIAN POPULATION

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Esophageal carcinoma is a major cause of cancer-related deaths among males and females in Iran. However, to date, the genetic alterations that accompany this disease in Iran have not been identified. The advent of comparative genomic hybridization (CGH) has opened a reliable way for the detection of all genomic imbalances in each tumor (including archival material) by only one single analysis. Not being dependent on mitoses, it overcame most limiting factors of classical cytogenetics. Chromosomal aberrations of 12 samples were analyzed successfully by CGH and their correlations with clinicopathologic staging and tumor progression were evaluated. In total, 33 gains and 15 losses were found in 12 tumor samples. Thus, the average number of gains and losses per patient were 2.75 and 1.25 respectively. Frequent gain abnormalities were found on chromosome arms 1p, 1q, 3q, 5p, 11q, 14q and 17q. Frequent deletions were found on chromosome arms 4q, 5q and 11q. Gain of 1p33-36 was significantly linked to nodal metastasis (stage IIB and III). Gain of 1p33-36 was also detected in grade 1 to grade 3 tumors and was coincided with the majority of detected chromosomal aberrations. These findings led us to propose a mutator picture, emphasizing the genomic unstabilising role for a gene, or genes, located on this segment of chromosome 1p. Consistent with our finding is the gene CASP9 (a member of apoptotic protease activating factors), mapped to chromosome 1p36.3-p36.1, that participates in caspase-3 activation which in turn leads to the degradation of DNase inhibitor and subsequently activation of caspase-activated DNase. It was also concluded that this abnormality might play an important role in the development of ESCC.

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ROLE OF GAMMA-TUBULIN IN MICROTUBULE REORGANIZATION DURING INDUCED DIFFERENTIATION OF HL-60 CELL

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Microtubules are the major components of cytoskeleton and play a crucial role in organizing eukaryotic cells. They determine cell shape and polarity; guide organelle and vesicle transport, and segregate chromosomes at mitosis. Microtubule dynamics depends on microtubule organizing center (MTOC) which nucleates microtubules from their heterodimers (α, β tubulin). Among the MTOCs, γ-tubulin is the major protein of these centers that show high homology with α and β tubulin. In this work we studied the role of γ-tubulin in reorganization of microtubules in normal and differentiated HL-60 cells. All trans retinoic acid (ATRA) was used as an inducer of differentiation and immunofluorescent microscopy was used to study the microtubule reorganization. We also examined γ-tubulin content and its activity using Western blot analysis and nocodazole test, respectively. The results revealed that, in comparison to undifferentiated cells, γ-tubulin content was increased following differentiation, and the differentiated cells had the ability to reorganize their microtubule network following nocodazole test, whereas undifferentiated cells did not show such ability. Our results suggest that γ-tubulin is an effector protein in differentiation of this cell line and could be a target in differentiation therapy of cancer.

p-470
THE EFFECT OF ERYTHROPOIETIN ON TELOMERASE ACTIVITY IN TF1 HUMAN MYELOID CELL LINE

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Telomeres are the physical ends of eukaryotic chromosomes. The maintenance of telomeres throughout many cycles of cell division requires the enzyme telomerase. Telomerase is repressed in somatic tissues, and that telomeres become shorter during somatic development. TF-1 is a human cell line, which shows many characteristics of erythroid cells. They can be induced to differentiate into more mature erythroid cells when cultured with erythropoietin (Epo). Leukemic cells are immature and are characterized by a block in the normal sequence of differentiation. To determine whether there is a linkage between telomerase regulation and lineage-specific maturation, we investigated the change in telomerase activity during the differentiation of TF-1 leukemia cell line. In this study we evaluated the effect of Epo on the cell growth, telomerase activity, and differentiation in the TF-1 erythroleukemia cell line. TF-1 cells were treated with Epo at concentrations of 1, 4, 10 U/ml. Treated cells were incubated for 96h at 37^{o} C in a humidified atmosphere of 5% CO2. Then the cells were collected and counted and assayed for proliferation with MTT and BrdU cell proliferation assays. Telomerase activity was assayed by a telomere repeat amplification protocol (TRAP-PCR ELISA) method. Significant decrease of telomerase activity and a marked increase in differentiation index were manifested by the cells at 4 and 10 U/ml dose of Epo treatment. Epo treatment did not have any significant effect on viability and cell proliferation after 96 h incubation. This data indicated that Epo as a natural cytokine that can decrease telomerase activity and induce differentiation in TF-1 cells. The early commitment to differentiation may therefore influence enzyme activity, changing telomerase to an inactive form.

p-471
CONSTRUCTION AND ANALYSIS OF THE VECTOR CONTAINING TBID (A PRO-APOPTOTIC GENE) INDUCED BY MUC1 PROMOTER, ESTROGEN AND HYPOXIA RESPONSIVE ELEMENTS

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The identification of promoters that are preferentially active in cancer cells is a promising gene therapy approach for the treatment of cancer. We examined the feasibility of using a tumor specific promoter of the MUC1 gene to direct therapeutic expression of a pro- apoptotic gene, tBid, specifically in human breast cancer cells. In many cases, breast cancer cells retain the expression of estrogen receptors and most solid tumors suffer from hypoxia. So, we studied the result of combination of estrogen response element (ERE) and hypoxia – responsive element (HRE) to MUC1 promoter in order to activate specific transcription of tBid gene in breast cancer cells. We confirmed the cloning of HRE/ERE cassette, MUC1 promoter and tBid gene in PCDNA 3.1/hygro by digestion and sequencing. Expression of this vector induced apoptotic cell death specifically in human tumor cells. Therefore, the MUC1 promoter is an excellent candidate for generating novel gene therapy vectors that control the expression of apoptotic genes specifically in breast tumor cells.

p-472
EVALUATING THE EXPRESSION OF BMI-1, A POLYCOMB GROUP REPRESSOR PROTEIN, IN BLADDER TUMORS

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The tissue homeostasis is the result of strict regulatory mechanisms, which control self-renewal, differentiation, and prevention of premature senescence and apoptosis of stem cells. Bmi-1, a polycomb group repressor protein, represses genes that induce cellular senescence and cell death, and can contribute to cancer when improperly expressed. Bladder tumor and non-tumorl samples were collected from Labafi-nejad hospital. RNA was extracted from each sample, reverse transcribed and amplified by PCR technique, using specific primers for Bmi-1 and β2-microglobolin, as an internal control. The production and distribution of Bmi-1 protein was

also examined by western blotting and immunohistochemistry techniques. To clarify the role of Bmi-1 in bladder tumors, we examined the expression of Bmi-1 in tumor and non-tumor samples. RT-PCR generated a 683 bp product, corresponding to the expected size of Bmi-1 amplified region. The identity of the amplified fragment was then confirmed by direct DNA sequencing. The mean of expression of Bmi-1 detected in tumor tissues was significantly higher than the non-tumor ones and there was also a significant correlation between the mean of gene expression with the stage of malignancy ($p < 0.05$). The expression of Bmi-1 at protein level was further confirmed by Western blotting and immunohistochemistry. The tumor suppressor locus Cdkn2a (Ink4a/Arf locus) codes for two proteins, p16ink4a and p14arf. Ink4a and arf are playing important roles in the retinoblastoma and p53 pathways, respectively. Bmi-1 is a potent repressor of both pathways and hence elucidating its role in tumorigenesis is very important. Here, we are reporting for the first time the expression of Bmi-1 and its correlation with malignncy in bladder tumors.

p-473
CONSTRUCTION OF A NEW T LYMPHOCYTE CHIMERIC RECEPTOR USING NANOBODIES AGAINST MUC1 TUMOR MARKER OF BREAST CANCER

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The specific activation of the immune system to control cancer growth has been a long-lasting goal in cancer immunotherapy. Employment of T cells for tumor therapy is an attractive approach. In order to redirect and enhance the ability of the patient's own immune cells to fight cancer, the chimeric receptor (CR) approach that endows lymphocytes with antibody specificity was developed. The CR consists of scFv that is linked through an extracellular linker to intracellular domains of lymphocyte triggering moieties. An extracellular spacer domain extends the distance of the antigen-binding domain from the cell surface and in our study we utilized CH2 & CH3 of IgG1. The transmembrane and cytoplasmic signalling domains are derived from CD28 and CD3 z-chain, which cause T cell activation. The most important problem of this type of immunotherapy is scFv, because it has poor solubility and stability in addition to having unstable linker in its structure. Part of the humoral immune response of camels and lamas is based on heavy-chain antibodies where the light chain is totally absent. These unique antibody isotypes interact with the antigen by virtue of only one single variable domain, referred to nanobody. Nanobodies have unique features as solubility, stability, and high specificity to their antigen and homology to human VH sequences. We developed a new construct, which expresses a CR containing nanobody against MUC1 instead of scFv. This construct might provide opportunities to develop a new generation of T cell therapy, hoping to solve some problems of T cell therapy.

p-474
A COMPARISON OF THE EFFECT OF SILYMARIN AND MESNA ON CYCLOPHOSPHAMIDE INDUCED-URINARY TRACT TOXICITY IN RATS

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Cyclophosphamide (CP) is used for the treatment of cancers and as immunosuppressive agent. This drug has several side effects including nephrotoxicity and induction of hemorrhagic cystitis. The metabolites of this drug, acrolein, mediates its side effects. In this study, effects of silymarin (as antioxidants produced from the seeds of Silybum marianum) and mesna (a chelator of acrolein) were compared. In addition, their effects were evaluated by co-administration of phenobarbital. The study was done in 7 groups of rats as follows: group 1, saline as a control; group 2, a single dose of CP; group 3, CP + mesna; group 4, CP + silymarin; group 5, CP + phenobarbital ; group 6, CP + phenobarbital + mesna and group 7, CP + phenobarbital + silymarin These drugs (except CP) were administrated daily for 4 days. In 7th day of the study, blood was collected and serum isolated. The kidneys and urinary bladders were removed and routinely prepared for histopathological examination by light microscope. Serum BUN, creatinine, sodium and potassium of all groups were measured by auto-analyzer. The results show that phenobarbital increased toxicity of CP and mesna and silymarin relatively attenuated its toxicity. It seems that these latter two compounds have protective but fairly different effects on CO toxicity.

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ESTABLISHMENT, CHARACTERIZATION AND DRUG SENSITIVITY OF A NEW EWING'S SARCOMA CELL LINE

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Ewing's sarcoma is one of the most malignant tumors in children and young adults. Only a few established cell lines of Ewing's sarcoma have been reported which made it difficult to study the biological features of these tumors. We have recently established a new Ewing's sarcoma cell line designated SS-ES-1 from the right thoracic tumor of a 16-year-old female patient. In this report, some characteristics of SS-ES-1 cells are presented. The cells were grown in DMEM media supplemented with 10% FBS and 100µg/ml streptomycin and 100u/ml penicillin in a humidified atmosphere with 7% CO2 at 37°C. The morphological studies were done and some photographs were prepared. The cells

were immunocytochemically characterized using a panel of monoclonal and polyclonal antibodies. Furthermore, the chemo-sensitivity of the cells to some anticancer drugs was assessed using MTT assay and IC50 values were reported. The morphological feature of SS-ES-1 cell line included poorly differentiated small round cells, which grew in multilayer with a doubling time of 19h. The cells have been grown continuously in culture for over 90 passages. The immunocytochemical staining demonstrated strong reactivity for CD99, neurofilament, P53, Ki67, Bax, estrogen receptor, cytokeratin and epithelial membrane antigen, and moderate reactivity for BCL2, MDR-1 and c-erb-B2 and no reactivity for c-erb-B1and GFAP. The SS-ES-1 cells displayed considerable sensitivity to vinbelastine (2±0.7picomole), which was followed by vincristine (0.3±0.12nM), doxorubicin (0.05±0.03μM), etoposide (0.64±0.28 μM) and cisplatin (0.67 ±0.45μM). In conclusion, SS-ES-1 cell line demonstrates unique cellular properties, which makes it a useful model for studying various aspects of the biology of Ewing's sarcoma.

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ACYLATION OF LIPIDS BY ARACHIDONIC ACID AND GENERATION OF LIPID SECOND MESSENGERS IN ANTI-CD3/CD28 CO-STIMULATED LYMPHOCYTES AT NORMAL AND DIVERSE FORMS OF LEUKEMIA

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The processes of quick (5 sec) and long-term (60 min) [14C]-arachidonic acid (AA) incorporation into the plasma membrane phospholipid and neutral lipid fractions in resting lymphocytes of patients with chronic lymphocytic, acute lymphoblastic and acute myeloblastic leukemias in comparison with the healthy people have been investigated. The regularities of diverse lipid second messenger (LSM) molecules formation in [14C] AA-pre-labeled lymphocytes at different time points (5, 10, 30 and 60 sec) of cell stimulation by anti-CD3 and anti-CD28 monoclonal antibodies cross-talking have also been investigated. The data demonstrate that there are regular defects in the mechanisms of lymphocyte lipids fatty acid content modification by AA as well as in the diverse AA-LSM generation processes in different forms of leukemia. Disturbances observed are identical for the three forms of leukemia only at the rapid, membrane-associated stage (5 sec) of lipid acylation and diverse LSM molecules generation is noted in response to external antigenic signal action. Violations, distinctive for each type of leukemia, differing from both normal and other types of the disease were observed at the relatively sustained stages of lipid acylation (60 min) and signal transduction (60 sec) processes. It is concluded, that alterations in some quick and reversible mechanisms of lipid modification and antigenic signal transduction processes, that are identical for all the studied types of leukemia, have a membrane-bound character and can serve as targets for chemotherapy.

p-477
STUDY OF LACTIC DEHYDROGENASE AND ALKALINE PHOSPHATASE LEVELS IN PATIENTS WITH HODGKIN\'S DISEASE AT THE CHILDREN'S MEDICAL CENTER OF TEHRAN

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Background: Hodgkin's disease is a neoplastic disorder originating in lymphoid tissue, usually occurring in all age groups, and initially localized, but subsequently spreads to contiguous lymphoid structures and ultimately disseminates to nonlymphoid tissues. The aim of this study was to measure lactate dehydrogenase and alkaline phosphatase levels in patients with Hodgkin's disease at the Children's Medical Center of Tehran. Methods: In the present study, the sera of all patients with the diagnosis of Hodgkin's disease referred to Children's Medical Center of Tehran were collected and tested for the activities of lactate dehydrogenase and alkaline phosphatase. Student t- test was used to analyse the data statistically. Results: The age range of patients was 2-13 years, with a median of 7.6 years, and a mean of 7.5 years. There were 51 males (73%) and 19 females (27%). Stages III and IV of the disease (advanced stages) occurred in 61.4% of the patients. Lactate dehydrogenase ($P<0.01$) and alkaline phosphatase activities ($P<0.05$) were more than the normal range. Conclusion: The results of the present study demonstrated increased levels of lactate dehydrogenase and alkaline phosphatase in advanced stages s'of Hodgkin disease.

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ANTI-CANCER ACTIVITY OF THE ELEMENTS OF LANTHANIDES GROUP: CYTOTOXIC ACTIVITY OF CERIUM IN A BREAST CANCER CELL LINE (MCF-7 CELLS)

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Lanthanides are a group of elements in periodic table known as rare earth metals. Up to now, there is no report on functional roles of lanthanides. Little is known about the effects of cerium in controlling cancer cell growth. The present study was to elucidate possible pathways for cytotoxic activity of cerium. For all assays, cells at approximately 60% confluence (105 cells) in microtiter plates were incubated with cerium at concentrations of 0.1, 1, 10 and 100μM, for 48h and 72 h, respectively. The growth of MCF-7 cells was tested by 3-(4, 5-dimethylthiazole-2-yl)-2, 5-biphenyl tetrazolium bromide (MTT) assay. Results were expressed as percent of untreated control. In MCF-7 cells, at the concentration of 0.1μM the most effect of cerium lanthanide was obtained and the viability percent of cells was reduced significantly both after 48 and 72 hours. The similarity of the size, coordination number and geometrical environment of iron and cerium ions, essential for the binding of these ions to proteins, is crucial for the combined metabolism of these two elements under

physiological conditions. Cancer cells are dependent on iron and cerium could occupy the iron sites and replace iron in iron containing proteins and reduce their function. This situation may cause cancer cells to take up the toxic element, cerium, instead of iron, and result in the retardation of their growth. Although the mechanism remains to be elucidated, our results indicate that at certain concentrations, cerium ion could inhibit the growth of cancer cells.

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NUCLEOSTEMIN GENE EXPRESSION IN TUMOR AND NON-TUMOR SAMPLES OF BLADDER

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Nucleostemin (NS) is a newly discovered GTP-binding protein expressed in stem cells and several cancer cell lines. It plays an important role in controlling self-renewal of stem cells, but its expression is shutting down upon cell differentiation. In the present study, we examined the expression of NS in bladder tumor and non-tumor tissues and evaluated its correlation with the state of malignancies. Bladder tumor samples were collected from Labbafi-nejad hospital. RNA was extracted from each sample, reverse transcribed and amplified by PCR technique, using specific primers for NS and b2m, as an internal control. Sub-cellular distribution of NS protein in tumor cells was also examined by immunohistochemistry. Our data revealed that NS is up-regulated in bladder tumors. Also, its relative expression in tumor tissues is significantly higher than in non-tumor tissues. Among different variants of NS, variant "1" is found in all tumor samples, whereas variant "2" and "3" are only detected in some of tumor samples. NS has a key role in controlling cancer cell proliferation. Moreover, differential expression of different variants of NS in tumor samples may indicate their distinct roles in cell proliferation control. So, it is probable to employ NS variants as a potential tumor marker for diagnosis and prognosis of bladder cancer. Also, inhibition of NS could be an effective strategy in reducing cancer cell proliferation rate.

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STUDY OF THE INTERACTION OF APIGENIN WITH DNA BY UV/VISIBLE AND FTIR SPECTROSCOPIC METHODS

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Apigenin is widely distributed in plant kingdom as polyphenolic compounds. Apigenin or 4, 5, 7-trihydroxyflavone is one of the most common dietary flavonoids. It is non-toxic and non-mutagenic, and is present in many fruits and vegetables including parsley, onions, orange, tea, wheat sprouts, etc. Several studies showed that Apigenin possesses anti-inflammatory, anti-tumor, antispasmodic, and antidiarrheal properties .The aim of this study was to examine the binding of Apigenin to calf thymus DNA in aqueous solution at pH 6.6- 7.1 Using a constant concentration of DNA with various molar ratios of drug/DNA (phosphate) of 1/40, 1/20, 1/10, 1/5. Fourier transform infrared (FTIR) and UV–visible difference spectroscopic methods were used to determine the binding properties of this flavonol including its binding constant, sequence selectivity and structural variations of drugs/DNA and complexes in aqueous solution . Spectroscopic evidence showed that interaction occurred mainly through Morin hydroxide and polynucleotide backbone phosphate group with an overall binding constant of $K=7.1x10^4\ M^{-1}$.

p-481
SERUM ANTIBODIES TO HPV16E7 AND GP96 FRAGMENTS AS BIOMARKERS IN IRANIAN WOMEN WITH INVASIVE CERVICAL CARCINOMA

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Cervical cancer is the second most frequent cancer among females worldwide. Certain types of human papilloma viruses (HPVs), mainly HPV types 16 and 18 have been recognized as major etiological factors for the development of cervical cancer. E7 is the major oncogenic protein produced in cervical cancer-associated HPV16. GP96, a 96 kDa glycoprotein, is the endoplasmic reticulum (ER) resident member of the HSP90 family, normally involved in stimulating the CTL response through either a direct or an indirect mechanism. Extracellular release of HSPs upon necrotic cell death and their modulated access at the surface of some cells can be considered as a putative 'danger' signal. In this study we present serum antibody response to HPV infections in patients with cervical cancer detected by Western blot and ELISA techniques based on recombinant HPV16 E7 and GP96 (N-terminal and C-terminal of gp96) proteins. These proteins were expressed in E. coli as a His-tag protein and purified by affinity chromatography using FPLC system. A total of 58 women serum samples were tested. The results showed that patients with higher antibody response to HPV16E7 had the same response at least to one of the gp96 fragments. Our data indicated that the prevalence of anti-E7, anti-NT-gp96 and anti-CT-gp96 antibody in patients suffering from adenocarcinoma is less than patients with squamous cell carcinoma (SCC). These data suggest that a combination of three recombinant proteins could be useful as a biomarker for diagnosis of SCC with different pathologies.

p-482
STUDY OF ANTI-TUBULOGENESIS ACTIVITY OF NIGELLA SATIVA SEED ON HUMAN BONE MARROW ENDOTHELIAL CELLS

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Angiogenesis, the formation of new blood capillaries from pre-existing capillaries and post-capillary venules, links with embryonic development and pathological conditions. Since angiogenesis is important in the pathogenesis of various diseases, the inhibition of angiogenesis, or anti-angiogenesis, is one of the promising approaches in their treatment. In this study we firstly extract Nigella sativa seed meal in deionized water overnight. Its anti- tubulogenesis activity studied in 3D microcarrier-based system. Different amounts of the aqueous extract were added to human bone marrow endothelial cells in the presence of endothelial growth factor in 3D fibrin gel. After 3 days anti- tubulogenesis activity was studied in comparison with control. In studied doses, aqueous extract of Nigella sativa showed strong anti-tubulogenesis activity. Aqueous extract inhibited endothelial cell migration and tubulogenesis in fibrin gels. These results show its anti-tumor activity and confirm its use in traditional medicine.

p-483
STUDY OF ANTI-ANGIOGENIC ACTIVITY OF GARLIC JUICE ON HUMAN BONE MARROW ENDOTHELIAL CELLS IN 3D FIBRIN GEL

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Angiogenesis, the formation of new blood capillaries from pre-existing capillaries and post-capillary venules, links with embryonic development and pathological conditions. Since angiogenesis has an important role in the pathogenesis of cancers, anti-angiogenesis is one of the promising approaches in their treatment. In this study we firstly grind fresh garlic bulbs and extract their juice. Anti-angiogenic activity of fresh garlic juice was studied in three-dimensional microcarrier-based system. Different amounts of garlic juice were added to human bone marrow endothelial cells in the presence of endothelial growth factor in three-dimensional fibrin gel. After 3 days, anti-angiogenic activity was studied microscopically in comparison with control. In studied doses garlic juice showed strong anti-angiogenic activity. Garlic juice inhibits endothelial cell migration and tubulogenesis in fibrin gel. These results show its anti-tumor activity and confirm its use in traditional medicine.

p-484
CXCL12/CXCR4 AND P53 EXPRESSION LEVELS IN PERIPHERAL BLOOD OF BREAST CANCER PATIENTS USING QUANTITATIVE REAL-TIME PCR (QPCR)

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Background: Chemokine receptor CXCR4 and its ligand CXCL12 are suggested to be involved in migration, invasion and metastasis of breast cancer cells. Mutation of the tumor suppressor gene p53 in breast cancer is associated with metastasis and aggressive clinical phenotype. objective: This study examined the expressions of CXCL12/ CXCR4 and p53 mRNA levels in peripheral blood and aimed to explore their significance in breast cancer patients. methods: Fifty women with breast cancer and 30 sex matched healthy cases were enrolled in this study. CXCL12/CXCR4 and p53 mRNA expression in peripheral blood were measured using real-time quantitative PCR (qPCR). results: Both CXCL12 and CXCR4 mRNA levels were significantly higher than those in normal controls. Also, we have found that the mRNA level of p53 significantly increased in comparison with normal group. conclusions: The increase of CXCL12/CXCR4 and p53 expression level in peripheral blood cells of breast cancer patients may be associated with the susceptibility and progression of breast cancer. The molecular profiling of blood cells may be used to identify therapeutic targets for cancer treatment.

p-485
POLYMORPHISM OF GLUTATHIONE S-TRANSFERASE P1 IN IRANIAN PATIENTS WITH ESOPHAGEAL SQUAMOUS CELL CARCINOMA AND NORMAL INDIVIDUALS

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Background & objectives: Esophageal squamous cell carcinoma (ESCC) ranks among the 10 most common human cancers worldwide. Glutathione S-transferases (GSTs) are a family of enzymes involved in detoxification of a wide range of chemicals, including carcinogens. GSTP1, the major GST isoform expressed in human epithelial cells of esophagus, has polymorphism at codon 105.This site lies in close proximity to the substrate binding site for the electrophilic molecules Materials & methods: To investigate the genetic association between GSTP1 polymorphism and susceptibility to ESCC, the GSTP1 genotypes were determined by PCR-RFLP analysis in 56 patients with ESCC and 25 healthy individuals. The expression of GSTP1 in ESCC as well as in normal tissue biopsies was determined by immunohistochemistry (IHC) Results: The frequencies of GST-P genotypes in the Iranian ESCC patients for Ile/Ile, Ile/Val and Val/Val were 73.2, 21.5 and 5.3%, respectively. Similar frequencies of genotypes were detected in normal individuals. No association was found

between GSTP1 genotypes with susceptibility to esophageal cancer although the expression of GSTP1 gene was significantly higher in ESCC samples as compared to normal tissues (p= 0.01) Conclusions: Our finding suggested that GSTP1 genotype plays very little role in developing ESCC in Iranian patients; however the GSTP1 expression level was found to be high in ESCC samples.

p-486
RELATIONSHIP BETWEEN TP53 MUTATIONS AND GASTRIC CANCER IN IRANIAN PATIENTS

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TP53 is a tumor-suppressing gene encoding a nuclear phosphoprotein which functions as a negative regulator of cell proliferation by arresting the DNA-damaged cells in G1 to S checkpoint. The TP53 causes mutations in diverse types of human cancers. The mutations of the TP53 and the loss of heterogeneity in chromosome 17p13.1 are associated with the tumors. The P53 protein interacts directly with the DNA via sequence-specific DNA-binding domain (DBD). More than 80% of TP53 mutations are in the hot spots and occur in the DBD domain and 90% of them are missense mutations. In the present study we detected the TP53 mutations occurring in the DNA-binding core domain of the protein (exons 5 through 8) in 43 paraffin block specimens, using PCR and single strand conformation polymorphism analysis (SSCP). The data showed that TP53 was mutated in the initiation of gastric carcinoma and even earlier in the non-neoplastic mucosa and increased in frequency concomitant with progression of gastric carcinoma development. The P53 immuno-reactivity was observed in 25%–87% of invasive gastric carcinomas and its mutations occur in 0–68% of the cases.

p-487
CYTOTOXICITY EFFECT OF AN ETHANOLIC EXTRACT OF NIGELLA SATIVA ON HUMAN KIDNEY CANCER CELL LINE (ACHN)

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Renal cell carcinoma (RCC) is the most common cancer of the genitourinary system. Annually, about 12500 people in the US die from RCC. Despite improved diagnostic and therapeutic methods, kidney cancer mortality rate has been increasing. In herbal medicine, Nigella sativa is widely used against cancers, and infectious and inflammatory diseases. The aim of this study is to investigate the effect of Nigella sativa ethanol extract on human renal cancer cell-line (ACHN). 70% ethanolic extract of Nigella sativa was prepared according to conventional methods. ACHN cell line was purchased from Pasteur Institute cell collection. Lethal effect of extract at 1.8, 2.5, 3 and 3.5 mg/ml concentration and cisplatin (1.5 microg/ml, as positive control) on kidney cancer cell-line (ACHN) was compared with normal control group using MTT assay and morphologic studies. All experiments were performed in a time-course of 24, 48 and 72 h. Morphologic studies showed that ethanolic extract at various concentrations and cisplatin have a cytotoxic action on ACHN cell line compared with control group after 72 h. MTT assay also confirmed these findings producing a significant reduction in absorbance ($P<0.05$) in both extract and cisplatin groups compared with the control. These results suggest that Nigella sativa extract similar to cisplatin has an anticancer effect on human kidney cancer cell-line (ACHN).

p-488
TELOMERASE INHIBITORY POTENCIES OF PLANT SECONDARY METABOLITES

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Because of crucial role in cell immortality, telomerase is a key target in cancer therapy. Normal somatic cells undergo telomere attrition and replicative senescence, while cancer cells escape mortality by up-regulating telomerase. Regarding to barely discernible telomerase activity in most normal cells, targeting telomerase looks to be a specific and safe strategy against cancer. It has been frequently shown that telomerase inhibition in human cell culture leads to telomere shortening and eventually highly specific death in cancer cells. Pure commercially available plant secondary metabolites especially natural alkaloids or their close derivatives were screened for their telomerase inhibitory potencies. For each compound IC50 was carefully assessed by different cytotoxicity tests including neutral red and MTT. Using a modified telomerase repeat amplification protocol (TRAP) assay telomerase activity was measured in cell extracts of 48 hours treated HepG2 and MCF7 with five different concentrations of each compound separately. Those compounds with moderately to highly telomerase inhibitory effect in the treated cultured cells, in comparison with controls, were selected for more investigation. Real time RT-PCR indicates down regulation of catalytic subunit of telomerase (hTERT) gene expression in treated cells with some of the compounds. Morphological changes and other features including induction of apoptosis/senescence suggest that at least some of the selected chemicals are valuable to be introduced as potential candidates for cancer therapy.

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THE HAEMOPOIETIC EFFECTS OF THROMBOPOIETIN ADMINISTERED POST CARBOPLATIN THERAPY COMPARED TO EITHER PRE - AND POST OR PRE- CHEMOTHERAPY.

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Thrombocytopenia is a major cause of morbidity following intensive chemotherapy for cancer. Over recent years, there has been an increasing use of platelet transfusion which, although generally efficacious to prevent severe hemorrhage, is associated with the risks of transmitting blood-borne diseases and of alloimmunization. Therefore, there is a clinical requirement for a drug that will reliably alleviate the thrombocytopenia associated with chemotherapy. Recombinant human thrombopoietin (rHuTPO) serves as a megakaryocyte colony-stimulating factor and predominantly acts on megakaryocyte progenitor cells, colony forming units-megakaryocyte (CFU-MK). We describe here the biologic rational and preliminary clinical use of rHuTPO that could be useful in the future for the management of thrombocytopenia in myelosuppressive chemotherapy. Balb/c mice (n=10) were treated with rHuTPO or placebo control by daily injection before (pre), after (post), or before and after (pre/post) carboplatin therapy. RHuTPO given post carboplatin therapy increased the number of platelets significantly ($P<0.01$). Furthermore, rHuTPO given pre/post carboplatin therapy also significantly increased the number of thrombocytes ($P<0.001$). Howeveranimals receiving rHuTPO pre carboplatin therapy exhibited significantly higher thrombocytopenia ($P<0.01$). We also showed the dose-dependent increase in the number of megakaryocytes on day 3 after a single injection of rHuTPO. These results suggest that post caboplatin therapy of rHuTPO appears to be more effective in enhancing platelet counts and HCT recovery following myelosuppressive chemotherapy than either prophylactic or pre-and post rHuTPO therapy.

p-490
PROLIFERATION INHIBITION AND INDUCTION OF APOPTOSIS IN RENAL CELL CARCINOMA CELL LINES (ACHN) BY THYMOQUINONE

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Thymoquinone (TQ) is a component of Nigella sativa, an herb that has been used for thousands of years for a variety of diseases including cancers. However, its effect on induction of apoptosis in renal cell carcinoma cell line (ACHN) was not clear. TQ-induced apoptosis was investigated in ACHN cells. ACHN cells were treated with different concentration of TQ (5-60 μM) for 24, 48 & 72h. Also, L929 cells were studied as normal controls. Cytotoxicity was determined using MTT assay and DNA fragmentation. Effects of TQ on the cell cycle were determined using flow cytometry. Apoptosis induction was assayed using annexin V and propidium iodine (PI) by flow cytometric analysis. Phosphatidylserine (PS) externalization is relatively increased in early process of apoptosis. It has high affinity for binding aprotein called annexin V. In this method, PS binding to annexin V-conjugated fluorescein isothiocyanate (FITC) and counterstaining with PI allows differentiation of necrotic and apoptotic cells. Cytotoxicity effect of TQ was time and dose-dependent. TQ (60μM after 24h), (30μM after 48h), (15μM after 72h) induced apoptosis and caused DNA fragmentation in ACHN cells. This concentration decreased the number of ACHN cells in S-phase and increased them in G1-phase, indicating cell cycle arrest at G1. This study showed that TQ induced early and late apoptosis and also necrosis in renal cell carcinoma without cytotoxicity on L929 cells. These results suggested that the tumor cytotoxic effect of thymoquinone on ACHN cells is mediated by a process involving apoptosis, necrosis and cell cycle arrest.

O-491
EFFECTS OF siRNA-MEDIATED IGF-IR GENE SILENCING ON COLON CANCER CELL LINES USING RADIOISOTOPES

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Colon cancer is one of the most common malignancies in the Western world. In both Europe and the United States, colon cancer accounts for 8 to 11% of all cancer deaths. Although the incidence of colon cancer was lower in Iran than in Europe and the U.S. in the mid-20th century, the incidence in Iran has now been rapidly increasing. Many genetic and epigenetic agents are involved in colon cancer development. Colon cancer cells generally posses the capability of overusing normal extracellular signaling for proliferation and/or anti-apoptosis to create growth advantages over the normal cells. Major players in extracellular signaling are the growth factor receptors. Among them, an activated IGF-IR is important for the establishment of a malignant cell phenotype, cell proliferation, cell mitogeny, cell size, cell growth, cell differentiation, and cell chemo- and radio-resistance and protection against apoptosis. Over-expression of IGF-IR in virtually all solid tumors, Makes colon cancer cells to synthesize and secrete IGF-I and IGF-II which can be incorporated with their IGF-IR membrane receptors or those of their neighboring cells causing the continuous proliferation of the tumor cells. It is suggested that the IGF-I and IGF-II and their receptors autocrine and paracrine proliferation cycling exist in colon cancer cells and may be the main reason for self-proliferation of the colon cancer tumor. For these results, IGF-IR seems to be a very promising target in colon

cancer therapy, although surgery, chemo- and radio-therapy are the main modalities of treatment for localized colon cancer. Considering that their efficacy is not so prominent in stage III patients (metastatic colon cancer) and because IGF-IR over-expression in cancer cells makes them chemo- and radio-resistance, IGF- IR gene silencing could be important as an aid in the therapy of this disease. Recent clinical trials have demonstrated. Some efficacy of anti-IGF-IR therapy in colon cancer. We therefore firstly investigated the effects of siRNA mediated silencing of IGF-IR in human colon cancer cells. RNAi, as the new technique of gene silencing, is a simple and effective tool superseding the gene knockout technique. RNAi has distinct advantages over other methods that inhibit gene expression, such as its high specificity, high efficacy, and high stabilization. The human colon cancer cell lines HT29 and SW480 were used in this research. Cells were cultured in RPMI 1640 medium supplemented with 10% FBS, at 37^{o} C with 5% CO2. Cell counting for relative cell viability was performed by trypan blue. Total RNA was isolated, electrophoresed and visualized by UV absorption. Results from RT-PCR demonstrated IGF-IR expression in HT29 and SW480 cell lines that are representative of tumor types. pkD IGF-IR siRNA was designed according to Tuschl and Ambion company guidelines and transfected into cell lines. Some results of this IGF-IR gene silencing including evaluation of IGF-IR down-regulation using IGF-I I-125, and also viability, growth, tumorigenesis potentiaal and radiosensivity changes will be presented. Finally we will discuss the benefits of siRNA targeting the IGF-IR, when used in combination with ionizing radiation.

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IL-13 SERUM LEVEL AND GENE POLYMORPHISMS AT POSITIONS -1512 A/C, -1055C/T AND +2044 G/A IN SOUTHERN IRANIAN WOMEN WITH BREAST CANCER

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Interleukin-13 (IL-13) is an immunoregulatory cytokine, secreted predominantly by activated Th2 lymphocytes. It has been postulated that deviation toward Th2 response down regulates effective tumor immune responses. IL-13, also, promotes growth or survival of certain types of tumors. In this study, association of three IL-13 gene polymorphisms at positions -1512 A/C and -1055 C/T in promoter and +2044 G/A in exon-4 was investigated in women with breast cancer and healthy controls. 305 women with breast cancer and 195 age-sex matched healthy individuals were recruited in this study. Genotyping of IL-13 gene polymorphisms were performed by PCR-RFLP methods. Serum level of IL-13 was determined by ELISA. As a result, no statistically significant differences were found in the frequencies of genotypes and alleles between patients and control group at all sites. However, higher ER expression was observed more frequently among patients harboring C allele at position -1512 as well as -1055. On the other hand, G allele at position +2044 has a significant correlation with left breast involvement. Haplotyping analysis revealed that ACA (-1512A/-1055C/+2044A) haplotype frequency is higher in normal women and CCA haplotype in women with breast cancer in comparison to another group. IL-13 serum level was undetectable in both patients and control subjects. In conclusion, results of this investigation suggest that the presence of CCA haplotype in IL-13 gene and the absence of ACA haplotype may be associated with susceptibility of women to breast cancer. -1512C and -1055C alleles may affect the progression of the disease in patients as well.

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INTERACTION OF THE ANTICANCER DRUG MORIN WITH DNA BY UV/VISIBLE AND FTIR SPECTROSCOPY

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Morin (3, 2′, 4′, 5, 7-pentahydroxyflavone) is one of the most common flavonols and occurs in Morus alba L. and many other herbs and fruits. As a widely distributed biological active compound, morin has attracted the attention of many researchers recently. The aim of this study was to examine the binding of Morin with calf thymus DNA in aqueous solutions at pH 6.6- 7.1 with constant concentrations of DNA and various molar ratios of drug/DNA (phosphate) of 1/40, 1/20, 1/10, and 1/5. Fourier transform infrared (FTIR) and UV–visible difference spectroscopic methods were used to determine the binding mode, binding constant, sequence selectivity and structural variations of morin/DNA and complexes in aqueous solution. Spectroscopic evidence showed that interaction occurred mainly through Morin hydroxide and polynucleotide backbone phosphate group with overall binding constant of $K= 5.99x10^{3}$ M^{-1}.

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INTERLEUKIN-13 SERUM LEVEL AND GENE POLYMORPHISMS IN IRANIAN PATIENTS WITH LUNG CANCER

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Lung cancer is a major cause of cancer deaths in men and women. Both immunocompetent and tumor cells secret cytokines, which play a key role in lung cancer immunity by developing type 2 cell-mediated immune response. IL-13 is an immunoregulatory cytokine affecting tumor immuno-surveillance by deviation of immune response from Th1 to Th2. The present study evaluates the association of IL-13 gene polymorphism at positions +2044 G/A and -1055 C/T in patients with lung cancer. One hundred and forty one patients and 113 control individuals were recruited in this study and

the control group was subdivided into smoker individuals and nonsmoker ones. Genotyping was carried out by PCR-RFLP assay and IL-13 serum detection was performed by ELISA method. No statistical differences were found in the frequencies of these two genotypes, their alleles and haplotypes in Iranian patients with lung cancer compared with healthy individuals. Serum level of IL-13 was not detectable, either.

p-495
THE APOPTOTIC EFFECT OF ACETAZOLAMIDE ON T47D CELL LINE

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Carbonic anhydrase plays an important role in the proliferation of cancer cells and is highly expressed in most cancers. We have previously reported that acetazolamide, as a member of sulfonamides, has an inhibitory effect on carbonic anhydrase in the cell-free condition. The anticancer effect of acetazolamide on T47D (a breast cancer cell line) was investigated using MTT test, fluorescence microscopy and flow cytometery. Our results have shown that acetazolamide demonstrates a growth inhibitory effect on T47D cell line in a time- and dose-dependent manner. Interestingly our techniques supported the possibility of induction of apoptosis in this cell line. Our future investigation will be focused on determination of the dependency or independency of the induced apoptosis process on the pathway of caspases in the cell.

p-496
ASSOCATION OF COX-2 SNPS WITH COLORECTAL CARCINOMA IN IRANIAN POPULATION

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Introduction: Epidemiological studies have implicated the role of the inducible form of cyclooxygenase (COX-2) in the pathogenesis of colorectal cancer; however, its role is not fully understood. Here, the association between Cox-2 SNPs and CRC (colorectal carcinoma) has been investigated. Materials and Methods: After obtaining the informed consent, DNA of 60 CRC patients and 100 healthy blood donors was extracted from whole blood using phenol-chloroform method. 12 different SNPs with maximum 1000 Kb distance weas selected to be genotyped using pyrosequencing method (rs4648298, rs3218625, rs5273, rs5277, rs20426, rs5270, rs3918304, rs689466, rs689462, rs2223626, and rs4648251). After determining the genotypes of so-called SNPs in 100 controls, Tag SNPs was selected using haploview software, and 60 cases were genotyped for tagged SNPs Results: After analysis of the 12 SNPs using pyrosequencing method, Tag SNPs were chosen by haploview 4.0 software. rs4648298, rs3218625, rs5277, rs689466, rs689462 were selected by heterozygosity of 7%, 1%, 2%, 7%, and 26%, respectively. In contrast to the other population we could not find the rare variant of the other SNPs in our population. After investigation of the cases for these 5 Tagged SNPs, we have observed significant differences between cases and controls in rs689466 SNPs heterozygosity (26% vs. 7% heterozygosity in controls vs. cases), respectively. Conclusion: There was a significant difference between cases and controls in SNP rs689466 A/G located in promoter region, showing its probable functional role in the regulation of Cox-2 gene.

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APOPTOSIS INDUCTION IN T47D CELL LINE IN THE PRESENCE OF SULFABENZAMIDE

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Regarding our previous studies about the inhibitory effects of sulfabenzamide on carbonic anhydrase activity (an enzyme which is highly expressed in most cancers), our new investigations have been focused on determination of some possible relationships between this drug and the programed cell death process. So, in our recent studies, the apoptotic effects of sulfabenzamide on T47D cells (a breast cancer cell line) was investigated using MTT test, fluorescence microscopy and flow cytometery. Interestingly our results indicated that the anticancer effect of this drug is probably through induction of apoptosis. Apoptopic effect of sulfonamide drugs is not investigated extensively. Therefore, it appears that our finding is novel. Caspase dependence of induced apoptosis is currently being investigated.

p-498
COMPARISION OF DIAGNOSTIC VALUE OF TELOMERASE ACTIVITY, PARATHYROID HORMONE, CARCINOEMBRYOGENIC ANTIGEN (CEA) AND CYFRA-21-1 IN LUNG CANCER PATIENTS

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Background and objectives: Lung cancer is the cause of many deaths worldwide. Many tumor specific biochemical markers were used in lung cancer diagnosis. The aim of this study was to compare the diagnostic value of telomerase enzyme activity, parathyroid hormone (PTH), carcinoembryogenic antigen (CEA), and Cyfra 21-1 in lung cancer patients. Materials and Methods: This was a case- control study and consisted of 50 lung cancer patients and 20 normal individuals as control. Telomerase activity was measured by TRAP assay, based on PCR- ELISA, in lung tumor biopsies. The serum levels of PTH, CEA, and Cyfra -21-1 were measured by commercially available immunoassay kits. Results: Telomerase activity had the highest sensitivity with 76.75 %. The mean value of Cyfra 21-1, CEA, and PTH were 58.25, 50, and 0%, respectfully. The highest value of sensitivity for Cyfra 21-1 in squamous cell carcinoma was 98% in lung cancer patients. The highest sensitivity of CEA was seen in

adenocarcinoma with 67%. Conclusion: It is speculated that telomerase activity and Cyfra 21-1 could be useful tools in diagnosis, prognosis, and screening lung cancer patients.

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THE EFFECT OF FOUR DIFFERENT TREATMENTS ON ESTRUS CYCLE AND CYTOSOLIC ESTROGEN AND PROGESTERONE RECEPTOR LEVELS IN DMBA-INDUCED MAMMARY GLAND CARCINOMA IN RAT

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Breast cancer is the most common cancer among women and about 80% of breast cancer express hormone receptors particularly estrogen (ER) and progesterone (PgR) receptors. Tamoxifen (TAM), CMF and ovariectomy are the most commonly utilized protocols in breast cancer treatment over the past 25 years. There are only a few studies correlating the effect of these therapeutic agents on estrus cycle and cytosolic ER and PgR levels. Sixty female Sprague Dawley rats were divided into six groups including negative control (no induction, no treatment), positive control (tumor induction, no treatment) and four different treatment groups. Tumors were induced by DMBA, 20 mg/2ml corn oil using gavage method. Trearment was started after palpable tumors were established. Duration of treatment was 17 days with 2-day intervals. Detection of estrus cycle phases was done by vaginal smear. ER, PgR, serum estrogen and progesterone were measured by RIA. Results showed that TAM produced more severe estrus cycle irregularity than the other groups. Expressions of PgR, ER were markedly reduced after all treatments but the reduction was much more pronounced in tamoxifen group. Weak reduction of ER and PgR and serum estrogen was demonstrated in combination therapy with CMF+TAM. There was a statistically significant correlation between reduction of tumor number and size with ER and serum estrogen reduction while in combinational therapy this correlation was weak and non significant. It is concluded that ER and PgR are good prognostic indicators in the four different treatment strategies.

p-500
ANTIPROLIFERATIVE AND APOPTOTIC EFFECTS OF THE NON-POLAR FACTIONS OF FERULA SZOVITSIANA ON HUMAN CANCER CELL LINES

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The use of traditional medicine is widespread, and plants still present a large source of novel active biological compounds with different activities, including anti-inflammatory, anticancer, antiviral, antibacterial, and antioxidant activities. The exclusively old world genus Ferula belongs to the family Umbelliferae, and has about 130 species distributed throughout the Mediterranean to Central Asia. The Iranian flora comprises of 30 species of Ferula, of which some are endemic. In this study, some non-polar fractions were separated from a chloroform extract of Ferula szovitsiana roots by column chromatography and preparative TLC. These fractions were evaluated for inhibition of some human cancer cells (HT-29 and HL-60), using MTT test. Cytotoxicity evaluation of the total extract and fractions revealed strong antiproliferative activity on cancer cell lines. Induction of apoptosis and the mode of action of fractions were determined by acridine orange/ethidium bromide double staining, invert microscopy and determination of caspase-3 activity. Chloroform extract and nonpolar fractions showed strong antiproliferative activity on cancer cell lines. According to the results Ff.3, Fr.5 and total extract showed the highest cytotoxicity on HT-29 cell line (IC50= 97.6, 202 and 220 μg/ml, respectively). Morphological studes showed chromatin condensation and detaching of HT-29 cells treated with chloroform extract and the fractions. It was also found that induction of apoptosis in HT-29 cells by these fractions is associated with caspase-3 activation. Our data indicate that non-polar fractions of Ferula szovitsiana can inhibit cell proliferation in human cancer cell lines through induction of apoptosis.

p-501
GLUTATHIONE S-TRANSFERASE ACTIVITY IN BREAST CANCER PATIENTS

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Overexpression of the glutathione S-transferases (GSTs) and their involvement in the detoxification of anticancer agents has prompted numerous investigations of the enzyme activity of human tumor tissues. Thus, we investigated the efficacy of using the GST activity as a biomarker of risk for breast cancer in patients and healthy treated individuals. In this study, GST activity was measured in the plasma of individuals who had previously been treated with both radiation and chemotherapy (n=23) and in the plasma, tumor tissue, and normal tissue adjacent to a tumor of patients with breast cancer (n=30). There were considerable differences in the investigated parameters among individual patients. Therefore we analyzed the paired samples of normal and cancerous tissues from the same individual. GST activity was assayed by measuring the absorbance of the conjugation reaction product of CDNB (1-chloro 2, 4- dinitrobenzene) by double- beam spectrophotometer. The GST activity was elevated significantly in tumors of the breast as compared to matched normal tissue from the same organ (p- value < 0.01). Plasma GST activity was significantly ($P<0.0001$) higher in breast cancer patients than those obtained from treated individuals. Correlation coefficient between GST activity in normal and corresponding cancer tissues was significant ($r = 0.47$, $p < 0.05$). Results revealed that measurement of plasma GST

Activity can be valuable as a tumor marker in breast cancer. The GST activity of primary breast tumors varied significantly with the treatment of the tumor.

p-502
APC GENE PROMOTER HYPERMETHYLATION AMONG IRANIAN COLORECTAL CANCER PATIENTS

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CpG island hypermethylation is a potential means of inactivating tumor suppressor genes. Many genes have been demonstrated to be silenced by aberrant promoter methylation in colorectal cancer (CRC). We are currently investigating the role of adenomatous polyposis coli (APC) tumor suppressor gene promoter hypermethylation in CRC development among cancer patients in Fars. To explore the APC gene promoter methylation status tumors and their nearby normal tissues were obtained from 86 sporadic CRC patients. After DNA extraction methylation status of APC promoter was investigated by methylation specific PCR. So far 47 samples have been analyzed and the frequency of aberrant promoter methylation was 12.7% in tumors. No APC methylation was detected for the adjacent normal tissues. It seems that inactivation of APC gene through aberrant promoter methylation plays an important role in CRC development in this region.

p-503
THE ROLE OF MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN 1 (MRP1) EXPRESSION AND ITS POLYMORPHISMS IN IRANIAN ACUTE LEUKEMIA PEDIATRIC PATIENTS: IMPACT ON TREATMENT OUTCOME

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Occurrence of cross-resistance to structurally and functionally unrelated drugs, called multidrug resistance (MDR), is a main cause of failure in the chemotherapeutic treatment of malignant disorders. Several mechanisms of MDR have been identified; one of these is the overexpression of adenosine triphosphate (ATP)-dependent membrane proteins that function as drug-efflux pumps. The multidrug resistance protein MRP1 is a member of the superfamily of ATP-binding cassette (ABC) transporters. MRP1, a 190-Kd protein, is encoded by the MRP1 gene located on chromosome 16p13 and has been shown to transport a broad range of organic substrates, such as glutathione (GSH) conjugates and other anionic conjugates. Our aim was to investigate the possible association between MRP1 gene expression level and its polymorphisms and clinical outcomes in Iranian pediatric patients. In a retrospective study, we analyzed samples obtained from 42 ALL pediatric patients similarly treated to assess whether the overexpression and/or function of this protein and also correlation between SNPs and overexpression correlate with treatment failure and therefore affects clinical outcome. In this regard total RNA and DNA were isolated from peripheral blood of ALL acute leukemia patients and ten healthy individuals. MRP1 gene overexpression was detected in 12 patients using Real-Time RT-PCR and compared to the type of response to chemotherapy. We also have investigated the associationn between MRP1 mRNA level and other clinical characteristics including cytogenetic subgroups and FAB subtypes. We also studied the effects of 4 SNPs (in exon 16 and 20) of MRP1 gene on its overexpression using PCR-SSCP methods followed by sequencing.

O-504
ROLE OF INTEGRINS ALPHA4 BETA1 AND ALPHA5 BETA1 IN CELL ADHESION AND MIGRATION OF LARGE CELL LUNG CANCER CELL LINE WITH NEUROENDOCRINE DIFFERENTIATION (MEHR-80)

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Integrins are αβ heterodimeric transmembrane proteins that attach the cell to the extracellular matrix (ECM), and anchor the cytoskeleton to the plasma membrane via focal adhesion formation. Members of the integrin family of cell adhesion receptors influence several important aspects of cancer cell behavior including adhesion, motility and invasiveness. Alternation of integrin expression in a number of different malignant diseases has been recognized. The integrins alpha5beta1 and alpha4beta1 both bind to ECM protein fibronectin (FN) and display distinct signaling properties. Considering the influence of alpha5beta1 and alpha4beta1 integrins in various important aspects of cancer cell behavior and the controversy between scientists over the role of these two integrins in metastasis and migration of cancer cells, we have investigated the role of alpha5beta1 and alpha4beta1 integrins in cell adhesion and migration of Mehr-80 (large cell lung cancer) cell line that has aggressive behavior. In this study, A375, A459, MRC-5 and HFLF in addition to Mehr-80, were cultured to investigate and compare the expression and adhesion behavior of alpha5beta1 and alpha4beta1 integrins in these cell lines by flowcytometry, adhesion, spreading and immunofluorescent assays. The results indicate that all of these cell lines except Mehr-80 express both integrins. Although Mehr-80 over expresses alpha5beta1 integrin and uses only this integrin for adhesion and focal adhesion formation, it does not express alpha4beta1 integrin. This result provides clear evidence for integrin alpha5beta1 to be positively correlated with Mehr-80 metastasis and might be an anti-tumour targe.

Genetic Engineering and Biotechnology

p-505
IN VITRO BIOACTIVITY ASSAY FOR POLYETHYLENE GLYCOL-CONJUGATED INTERFERON-ALPHA

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PEGylation is a process of attaching the strands of the polymer Polyethylene glycol (PEG) to proteins. PEGylated interferon-α formulation increases its molecular size, solubility and shields the metabolic sites. It increases plasma half-life and in vivo stability. So it can enhance therapeutic efficacy. The in vitro antiviral activity of PEG–IFN-α was determined by cytopathic effect assay using Hela cells challenged with vesicular stomatitis virus (VSV) and compared to native (unmodified) form. Bioassay procedure: Hela cells were grown in RPMI 1640 supplemented with 10% fetal bovine serum.100 µL of cell culture at a density of 10^6 cells/ml was added to each well of 96- well microtiter plate. PEG–IFN-α and native IFN-α were diluted in the medium with varying concentrations and added to separate wells in a volume of 50 µL in all rows except two rows. After incubation for 24 hours at 37°C, a confluent cell monolayer was obtained and 50 µL of diluted VSV solution in RPMI without fetal bovine serum was added in each well of all rows except one row. After an additional 24 hour of incubation, the supernatants were decanted and the cells were stained with 2% neutral red solution. The optical density was read at 540 nm using a micro well plate reader. The potency of PEG-IFN was determined by comparing the dose of PEG-IFN with that of the native IFN, which gave 50% protection to infected cells. Result: The average, specific antiviral activity of PEG-IFN was approximately 10% of the native IFN-α. For in vitro antiproliferative activities of IFN-α and PEG-IFN, EC50 (effective concentration, where 50% response is observed) were 0.047 and 0.510 ng/mL, respectively.

p-506
ORGAN SPECIFIC EXPRESSION OF HUMAN CALCITONIN GENE IN TRANSGENIC POTATO TUBER

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Molecular farming is a new and promising industry involving plant biotechnology for the production of pharmaceutically valuable proteins in plants. Potato (Solanum tuberosum) is a globally important crop producing high yields of nutritionally valuable food crop and as a potentially significant source of interest compounds. Human Calcitonin (hCT) is a peptide hormone which is secreted by the parafollicular cells of the thyroid gland. It plays an important pharmaceutical role in treatment of osteoporosis, hypercalcemia and Paget's disease. So far hCT for therapeutic use has been industrially produced by completely chemical synthesis. The high costs of the synthetic hormone and the increasing therapeutic need for hCT promote research activities in order to develop a biotechnological process for the recombinant production of hCT. In an attempt to produce of recombinant hCT we used synthetic hCT gene for organ specific expression in transgenic potato tuber under the control of class I patatin promoter. Expression cassette was inserted between left and right border of the binary vector Bin19. Potato tuber discs were transformed using Agrobacterium tumefaciens carrying pBin19-ppIhCT and transgenic plants were selected on medium containing kanamycin (Km). After regeneration, tubers were obtained from tuberization MS medium. Total extracted plant protein was used for determining of human calcitonin expression in transgenic tuber by ELISA. Results showed that the level of calcitonin in Kardal variety 1472/8585 pg/g fresh tuber. This is the first report for organ specific expression of hCT gene in potato tuber.

p-507
BACTERIAL EXPRESSION OF APO-CYTOCHROME-C: TOWARDS STUDYING CYTOCHROME-C ASSEMBLY

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Cytochrome-c is normally produced with a heme prosthetic group that is attached post-synthetically through two thioether bonds between the heme vinyl groups. As a result, it is very difficult to remove the heme and study the mechanism of heme binding. At present there is no convenient method for preparing apo-cytochrome-c and a recombinant apo-cytochrome-c has not been produced. With the aim of studying cytochrome-c assembly, an expression system for apo-cytochrome-c has been developed. We introduced a DNA sequence downstream from the gene that encodes green fluoresent protein (GFP) in PRSETbGFP that adds the hexapeptide for thrombin recognition/cleavage at the C-terminus of GFP. Then we fused the gene that encodes yeast cytochrome-c (CYC1) downstream from the thrombin cleavage site in PRSETGFPthr. This expression plasmid is called PRSETGFPthrCYC1. To make ensure that there are no spontaneous mutations or other errors in open reading frame, we sequenced the plasmid, and then expressed the fusion protein of cytochrome-c with GFP in E.coli. Ecoli is unable to put heme into apo-cytochrome-c unless the enzyme cytochrome-c heme lyase is expressed at the same time, so there is no chance of heme binding to the apo protein in E. coli. The fusion protein containing GFP has been provided a convenient means of locating the apo protein because GFP has a bright green color. After purification, the GFP has been removed with a protease.

O-508
ISOLATION AND CHARACTERIZATION OF AN AP2/ERF DOMAIN TRANSCRIPTION FACTOR (TADREB) IN DROUGHT STRESS FROM WHEAT

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Plant growth and productivity are affected by abiotic stresses, such as drought. Plants respond and adapt to this stress at the physiological and biochemical levels. Recent studies are focused on plant molecular responses to water stress like expression of inducible genes such as transcription factors. AP2/EREBP is a family of plant-specific transcription factors containing the highly conserved region of about 58-70 amino acids, the so-called AP2/ERF domain. DRE-binding proteins (DREB) are a subfamily of AP2/EREBP family. Members of

DREB subfamily play a crucial role in the tolerance of plants to abiotic stresses. The aim of this study was isolation and characterization of a member of DREB in Iranian wheat germplasm. Seedling of Iranian bread wheat (Triticum aestivum L.), Sardari cultivar was grown in growth chamber for 10 days in hydroponic culture. Drought stress was induced by poly-ethylene glycol 6000. Total mRNA isolation and cDNA synthesis were carried out from intact seedlings. An EST of DREB was isolated by specific primers via PCR. Obtained partial cDNA sequence (TaDREB) with 645 nucleotides was analyzed using internet software. The presence of conserved AP2/ERF domain in our sequence was confirmed by motif search tools such as CDART, CDD, SMART, Pfam and PROSITE. Alignment and phylogenic tree was drawn using DNAMAN program and it showed that the sequence had about 85-98% similarity with other T. aestivum DREB sequences which are in NCBI GenBank. The sequence was cloned in pTZ57R/T plasmid and also deposited in NCBI GenBank databases under the accession number ES466900.

p-509

ISOLATION, SCREENING AND TRANSFER OF CELLULASE GENE FROM BACILLUS SUBTILIS TO LYSINE PRODUCING MUTANTS OF BREVIBACTERIUM FLAVUM

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Microbial enzymes (e.g. cellulases) are very important in industry for digestion and removal of undesired polymers. *Bacillus subtilis* has been reported to secrete considerable quantities of cellulases and can successfully digest cellulosic materials into glucose. *Brevibacterium flavum* is industrial producer of lysine but it lacks detectable extracellular cellulase activity. B. flavum, used for lysine production in this study, was grown on nutrient agar medium at 30^{o} C for 24 hours. The mutagen, N-nitroso-N-ethylurea (ENU) was used for hyper-expression of lysine. Cells were exposed to 35 Mm ENU for 5-30 minutes. After washing the bacterial pellet with sodium citrate buffer and growing on seed culture medium, lysine production was enhanced up to 1.25 g/L in the culture exposed to the mutagen for 5 minutes as compared to 0.81 g/L, produced from wild type bacterial cells. Optimization of different growth parameters showed that the optimum condition for bacterial growth was 30^{o}C temperature, pH 7.0, 2% methionine and threonine, 20 g/l glucose as sole substrate for bacterial growth, 0.2-1.2 g/l lactate as growth enhancer, for 24 hours incubation. In the next step of study, *Bacillus subtilis* was grown on Lauria-bertani growth medium and 1.92 µg/µl of DNA was isolated by using genomic DNA isolation kit. After RNase treatment, the amount of DNA obtained was 1.7 µg/µl. The Gel documentation for time course experiment (15-180 minutes) showed that BamH1 digestion of DNA for 180 minutes was the most effective way. Rapid estimation of DNA concentration with ethidium bromide dot quantification procedure yielded 150.25 ng/µl of DNA. Ligation into BamHI digested pUC19 vector was carried out with rapid DNA ligation and transformation kit. After DNA transformation in E. coli by heat shock method, DNA libraries were screened on agar plates with 12.48 ml/l ampicillin for cellulase genes. We will transform the construct to B. flavum mutants in future.

p-510

CLONING AND EXPRESSION OF HEPTAD REPEAT REGION 1 OF THE NEWCASTLE DISEASE VIRUS FUSION PROTEIN USING PET32A (+) VECTOR

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Background: Newcastle disease virus (NDV) causes a highly contagious respiratory, neurological, or enteric disease in chickens. The disease is prevalent worldwide and in many countries the disease remains one of the major problems affecting existing or developing poultry industries. The fusion (F) protein is a protein of virus involved in mediating fusion of the viral envelope with cellular membranes. Heptad repeat region 1 (HR1) peptide of F protein can be used for vaccination, immunological diagnosis and treatment of infected chicken. To design antiviral and anticancer drugs it can also be used as analyzing and investigational model to study the effects of HR peptides on fusion activity. Methods: After obtaining virus from poultry we extracted NDV (NR43) RNA using RNX kit (Cinagen, Tehran, Iran), performed RT-PCR technique, and then HR1 cDNA was cloned into pET32a(+) expression vector and transformed into E.coli BL21(DE3) bacteria by heat shock. The expression of the recombinant HR1 gene was induced at 30º C temperature using IPTG in 1 mM concentration. Results and conclusion: HR1 amino acid and nucleotide sequence was aligned using Blast software and deposited at GenBank (AY678224). The obtained peptide was analyzed by SDS-PAGE and Western-Blotting. Finally, the concentration of the expressed recombinant HR1 peptide was about 2.4 mg/ml.

P-511

PRODUCTION AND SELECTION OF ENGINEERED ANTIBODIES AGAINST TUMOR SURFACE ANTIGEN P185

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Background: Recent advances in antibody engineering has made possible the production of human recombinant antibody fragments in phage display vectors in the form of single chain fragment variable (scFv). The genes encoding the human heavy (VH) and light (VL) chain variable domains are amplified and randomly assembled together and then cloned into the minor coat protein gene (g3p) of M13 filamentous

bacteriophage. The resulting library of scFv is expressed on the phage surface as g3p fusion proteins. It is possible to select high affinity scFv by panning against specific antigens. Here we describe the selection of specific clones against p185 which is significantly overexpressed in several cancer cells . Methods: A phage antibody display library of single chain fragment variable (scFv) was produced and applied to develop anti-p185 antibodies. To enrich for specific scFvs, the phage antibody was panned against p185 epitopes. BstN1 fingerprinting differentiated a number of clones. The specific clones were selected and tested by ELISA. Results: PCR products of selected clones from the library showed expected size in agarose gel electrophoresis and the fingerprinting revealed a large and diverse library. Successful panning selected specific clones against p185 epitopes which were positive as assayed by ELISA. Conclusion: The high affinity libraries selected against p185 suggest a good targeting index. As minimal antigen binding fragment, the scFv is favored for phage display techniques and phage antibody library construction. The anti-p185 engineered antibodies which are wholly human in origin could be applied in diagnosis and treatment of p185 overexpressing cancers in order to overcome several drawbacks of murin MAb.

p-512
PROTEIN PROFILING TECHNIQUES TO IDENTIFY DIFFERENCES BETWEEN WILD AND RECOMBINANT FORMS LEISHMANIA MAJOR

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In order use new vaccine candidates in clinical trial, a genetically modified Leishmania major parasite has been developed by inserting a thymidine kinase gene (tk), causing sensitivity to ganciclovir (GCV), and a cytosine deaminase gene (cd), causing sensitivity to 5-fluorocytosine (5-FC). Comparative proteomics analysis has been used to investigate effects of this genetic modification on protein expression profile of the parasite. The wild and recombinant leishmania Promastigotes were cultured separately. Cells were harvested at late log phase and proteins were extracted by lysis buffer. Three different approaches were applied for comparative analysis. Reverse phase high performance liquid chromatography (RP-HPLC) was used to provide the general protein profile pattern of whole cell lysate of two samples. The obtained chromatograms showed apparent differences in peptide/protein contents. The membrane proteins were extracted separately and analyzed by 16-benzyldimethyl-n-hexadecylammoniumchloride (16-BAC/ SDS-PAGE) which showed no significant differences between two samples. Whole cell lysates were undergone a polishing step by trichloroacetic acid (TCA) precipitation and then analysed by two-dimensional gel electrophoresis (2DE) using 17 cm immobilized pH gradient (IPG) strips with nonlinear pH 3-10 in first dimension and 12% SDS-PAGE in second dimension. Gels stained by silver nitrate and image analysis with PG200 software showed a few quantitative differences of protein expression between two samples.

p-513
CONSTRUCTION OF AN EXPRESSION VECTOR CARRYING MOUSE PEROXISOMAL PROTEIN GENE (PEP) WITH GST LABEL

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Peroxisomes are ubiquitious, single membrane bound organelles of eukaryotic cells. They have various functions that differ depending on the species and cell type, as well as the environmental or developmental conditions. Peroxisomal matrix proteins are synthesized on free polyribosomes in the cytosol and are imported into the organelle via one of either two pathways requiring two evolutionary conserved peroxisomal targeting signal (PTS) sequences. One of the peroxisomal matrix proteins, termed Peroxisomal Protein (PeP) which its gene encodes a predicted protein of 209 residues with a calculated molecular mass of 23,319 Da, has been cloned in mouse in 2002. Studies have shown that PeP expression in mouse embryo is increased considerably during myoblast formation and skeletal muscle differentiation, but not in other tissues like heart and brain. The reason remains unclear. We have started to sub-clone this gene in appropriate prokaryote expression vector to purify PeP protein for further biochemical analysis and identifying related protein. To construct a chimeric cDNA composed of GST with PeP cDND, we used a PGEX plasmid containing GST gene. PeP cDNA was amplified and inserted downstream of GST gene in this plasmid and purification of the protein is in progress now.

p-514
BCR-ABL SILENCING USING SPECIFIC SMALL INTERFERENCE RNA (siRNA)

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Chronic myelogenous leukemia (CML) is a human malignancy marked by the presence of a distinct cytogenetic abnormality which results from a translocation between chromosomes 9 and2 2, t (9; 22), and known as Philadelphia chromosome. This translocation causes aberrant expression of Bcr-Abl a constitutively active tyrosine kinase that has been linked to the pathogenesis of CML. Bcr-Abl is thought to promote malignant transformation by altering cellular adhesion properties, stimulating mitogenic signaling pathways, and inhibiting programmed cell death (apoptosis). Bcr-Abl prevents apoptosis through inhibition of mitochondrial cytochrome C release or inhibition of caspase activation after cytochrome C release. Therefore it appears that Bcr-Abl silencing can induce apoptosis in CML cells. The aim of this study was to silence Bcr-Abl gene by using a specific small interfering RNA (19-22 nucleotides) in cultured K562 cell line. By using mRNA sequence of Bcr-Abl from NCBI, various small interfering RNA targeting Bcr-Abl mRNA were designed and blasted in NCBI, then the suitable

small hairpin RNA was sellected and related DNA synthesized and cloned to pRNAH1.1/Neo vector using Bam HI and Hind III restriction enzymes. Recombinant vector delivered to E. coli (DH5α). The correct transmission of expression vector was confirmed by using of restriction enzymes and electrophoresis. Expression vectors which were isolated from E. coli (DH5α), transfected to K562 cell lines by electroporation procedure and incubated in culture media. Gene silencing was investigated by monoclonal antibody against Bcr-Abl gene product. Restriction digest confirmed correct inserted gene product. The extent of gene silencing by western blot showed significant reduction in gene products. This results, suggest that small interfering RNA is a suitable for gene silencing in cancer and viral infection.

p-515
CLONING OF THE GENE ENCODING THE SEFA FIMBRIAL ANTIGEN OF SALMONELLA ENTERITIDIS (E3) IN THE PTZ57R/T VECTOR.

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The emergence of *Salmonella enteritidis* as an important food-borne pathogenesis in humans, demands the development of novel intervention strategies. It is generally accepted that fimbriae are important factors in bacterial survival and persistence in the host. Salmonella enteritidis fimbriae antigens are useful as an antigen for immunoassay diagnosis of *Salmonella enteritidis* infection or as evidence of infection and can also be helpful in infection treatment. Progress in the molecular biology techniques created opportunities for producing such antigens. This study is directed towards the method of amplifying and cloning the sef-A gene. Strains used for these studies were *Salmonella enteritidis* (E3), which were collected from Kermanshah. Chromosomal DNA was extracted by boiling method and PCR reaction was performed and a single band of 511 bp was amplified by sef-A-F and sef-A-R primers. The resulting PCR product was inserted into the cloning vector (pTZ57R/T). In order to amplify the recombinant plasmid, E.coli DH5α bacteria was transformed with sefA-pTZ57R/T. Recombinant clones were identified by blue/white selection and purified recombinant plasmids were indicated by an alkaline lysis procedure. Identity of the sefA-pTZ57R/T product was confirmed by RFLP and sequencing. Nucleotide and protein alignment with BLAST software showed that the sequence of sef-A gene derived from Salmonella enteritidis (E3), which was cloned in the pTZ57R/T vector, was 99% identical to that of the Genbank (L11008). Furthermore, they differed only in two nucleotides, and one amino acid. The cloned sef-A gene from Salmonella enteritidis (E3) was submitted to the NCBI Genbank (EF553334).

p-516
LIPOFECTION OF BOVINE SPERMATOZOA TO CARRY EGFP TRANSGENE INTO OOCYTES

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Spermatozoa from numerous species, including bovine, can bind and take up foreign DNA and transfer it to the embryo. Genetically transformed animals can be generated using this method with variable degrees of success. The present study investigated whether EGFP could be transferred into bovine genome using sperm transformation and lipofection. Motile bovine spermatozoa were isolated through a Puresperm gradient, and resuspended at a concentration of 1×10^6/ml in SP-TALP medium. One microlitre of lipofectamine was added to 50 µl of Opti-MEM medium and combined with 50 µl of Opti-MEM containing 1µg of pEGFP-C1. The pEGFP–liposome mixture was incubated at room temperature for 1 h and then added to the spermatozoa (1×10^6/ml). For incubation 400 ng of pEGFP-C1 was incubated with sperms at 37°C for 1.5 hours. Sperm motility after incubation and lipofection methods was 65 and 10 percent, respectively. We found that lipofectamine: DNA amount ratio has a great effect on sperm motility and use of a 12:1 ratio was so harmful for sperm motility. The lipofected sperms DNA was used for ICSI, but all of the incubated naked-DNA was used for normal IVF with in vitro matured bovine oocytes. The expression of EGFP in early, late, and hatched blastocysts placed in a micro drop of PBS medium was observed by a fluorescence microscope. Expanded blastocyst from IVF and ICSI oocytes were 40 and 50 percent, respectively. The GFP expression was shown in both lipofected and incubated groups. There was some evidence that some of blastocycts were mosaic for GFP transgene.

p-517
AGROBACTERIUM-MEDIATED TRANSFORMATION OF POTATO (SOLANUM TUBEROSUM L.) WITH CRY1AB GENE FOR ENHANCED RESISTANCE TO POTATO TUBER MOTH (PHTHORIMEAE OPERCULELLAE)

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Potato tuber moth (Phthorimaea operculella) is one of the major insect pests of potato plants. One of the best and usual methods for biological control of this pathogen is transferring of Cry1Ab gene from B.t (Bacillus turengiensis) to potato plant. Cry1Ab gene encodes a crystal protein that causes perturbation of Lepidopteran insects digestive apparatus and eventually the insect dies. For Agrobacterium-mediated transformation, Cry1Ab gene was separated from GEMCry1Ab plasmid and placed under very strong promoter (CaMV35s), from Cauliflower Mosaic virus, within a suitable vector. Bin19 plasmid was selected as a binary vector that contains kanamycin resistance marker gene. At the first step, CaMV35s (871 bp) from BI121 plasmid was separated by

means of digestion reaction using two restriction enzymes, BamH1 and HindIII. CaMV35S was inserted in MCS region of Bin19 binary vector using T4-DNA ligase. In the next step, Cry1Ab gene with nos-terminator (2.1 kb), was separated from GEMCry1Ab plasmid, using BamH1 restriction enzyme and was inserted into T-DNA region of Bin19 plasmid in downstream of 35s promoter. The fidelity of insertion of Cry1Ab gene has been tested with polymerase chain reaction (PCR). Furthermore, the insertion of promoter and Cry1Ab gene has been confirmed using restriction mapping method. Constructed vector was named pBCCry1Ab and was cloned in E.coli (X1LBlue strain) and then was transformed in to Agrobacterium tumefaciensis using freez-thaw method. For verification of presence of plasmid, PCR analysis was established. In this study, by using internodes, from in vitro grown potatoes and a selectable marker, neomycin phosphotransferase (NPT II), we were able to transfer Cry1Ab gene into potato by Agrobacterium-mediated transformation method.

p-518
USE OF A BACTERIALLY DERIVED HUMAN FACTOR IX FRAGMENT (GAL DOMAIN) FOR DEVELOPMENT OF POLYCLONAL ANTI HUMAN FACTOR IX

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Haemophilia B is an X-linked recessive bleeding disorder caused by a deficiency or malfunctioning of coagulation factor IX (FIX). The hemophilia B patients are treated currently by infusion of plasma derived hFIX which is not always efficient. In some cases development of anti-hFIX antibodies (alloantibody) inhibits the activity of the infused hFIX. The hFIX alloantibodies are directed against domains containing either the gama-carboxyglutamic acid residues (Gla domain) or protease domain. In order to produce specific anti-hFIX antibody a bacterially derived hFIX sub-fragment can be used as an antigen, able to neutralize the alloantibodies. With the aim of over-expression of an epitop-containing of hFIX and development of its polyclonal antibody, a 540 bp DNA fragment coding for the hFIX Gla-domain, was amplified and cloned in a T7-based E. coli expression vector. A BL21 (DE3) strain of E. coli transformed with the recombinant plasmid was subjected for expression analysis. After IPTG induction, an overexpression of a protein with the expected size among the recombinant bacterial proteins was documented by SDS-PAGE analysis and Immunoblotting using anti-hFIX antibody. After optimization of the growth and induction, the over-expressed protein was eluted from a preparative polyacrylamide gel and used for development of specific anti-hFIX antibody in rabbit. The polyclonal antibody produced in this work is able to detect the normal human factor IX. Considering the importance of hFIX and its related investigations, both produced hFIX antigens and its corresponding antibody are important for the studies for the neutralization of the hFIX-alloantibodies as well as hFIX production.

p-519
CONSTRUCTION AND EXPRESSION OF RECOMBINANT FUSION PROTEINS OF THE B SUBUNIT OF E.COLLI SHIGATOXIN AND AAF ADHESIN

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Diarrhea is the major public health problem in many parts of the world. Different pathotypes of E. coli have been recognized. Shigatoxin producing E. coli (STEC) are among these pathotypes, causing bloody diarrhea. Shigatoxin is a bacterial toxin produced by this group of E. coli, the A subunit of toxin inhibits protein synthesis and B subunit is homopentamer responsible for toxin binding into target cells. Enteroaggregative E. coli (EAEC) is another pathotype of diarrheagenic E. coli and its mechanism of action is not fully known. However they express adhesins (AAFs) that binds to the receptor on the cell surface and colonizes the gut. In the present study the B subunit of Shiga toxin has been fused genetically to gene encoding AAF adhesin of EAEC (B-AAF) and expressed in E.coli. The B subunit and AAF/I encoding genes were amplified using specific primers and the resulting genes were fused by PCR. The fused gene was cloned in the pBADgIII/A and transformed into competent Top10 cells. Hence the construct was expressed and protein was analyzed on SDS-PAGE and confirmed by western blot. The maximum protein expression was obtained by addition of 0.2 - 2% of arabinose for 4 h. The results showed that the fusion protein containing the B subunit and AAF protein was successfully constructed and expressed. Hence the protein with correct conformation will be used for further immunological studies.

p-520
INCREASING ANTIBIOTIC PRODUCTION BY CONSTRUCTING RECOMBINANT PMA::HYG VECTOR

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Antibiotic production is under the control of an array of regulatory signals in hierarchical manner. The pathway-specific transcriptional regulators activate or negatively regulate the expression of the antibiotic biosynthetic genes. The clinically used beta-lactamase inhibitor clavulanic acid is produced by fermentation of *Streptomyces clavuligerus*. ClaR is the pathway-specific transcriptional regulator for genes involved in clavulanic acid biosynthesis. Infection of beta-lactamase positive bacteria is inhibited by Clavulanic acid in combination with beta-lactam antibiotics. Since constructing appropriate recombinant vectors make it possible to increase the amount of clavulanic acid production following *S. clavuligerus* genome manipulation, we have cloned claR gene in pMA::hyg vector (vector of *S. clavuligerus*) and detected the transformed Escherichia coli colonies by PCR method and

enzymatic digestion. After preparing E.coli competent cells, they were transformed by pMA::hyg vectors and grown in media containing ampicillin as selective marker. Following spore preparation from *S. clavuligerus* 738, cultures for isolation of chromosomal DNA were made by inoculating spores into YEME medium. The claR gene was amplified by RFLP-PCR from *S. clavuligerus* genomic DNA and confirmed by Nested PCR. Another method was Colony-PCR for claR gene amplification. Highly specific designed forward and reverse primers contained BamH1 and Xba1 restriction sites. In order to produce recombinant vectors, amplified claR gene and propagated pMA::hyg vectors were ligated using appropriate restriction enzymes. Finally E.coli competent cells were transformed with recombinant vectors. The future work includes introducing the recombinant vectors into S. clavuligerus.

O-521
EXPRESSION OF GENE ENCODING TOXOPLASMA GONDII MAJOR SURFACE ANTIGEN (SAG1) USING PCDNA3 IN CHO CELL CULTURE

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Toxoplasmosis, caused by a protozoan parasite, Toxoplasma gondii, is widespread throughout the world and is one of the major medical and veterinary issue. In recent years significant progress has been made in the identification of candidate vaccine which can induce a protective response. SAG1 is the immunodominant antigen of T.gondii and therefore is a potential diagnostic tool and/or subunit or DNA vaccine against toxoplasmosis. In this research first the SAG1 coding sequence was amplified by PCR from genomic DNA of T.gondii RH strain and cloned into HindIII/EcoRI sites into MCS of pTZ57R/T and transformed into competent E. coli. The recombinant plasmid pT-SAG1 was detected by restriction analysis with EcoRI and HindIII, PCR and sequencing. Then SAG1 gene was subcloned from pT-SAG1 with linkers to join to the HindIII and EcoRI sites of the pcDNA3 (Invitrogen,USA) to produce recombinant eukaryotic expression vector pcSAG1. The plasmids with the correct insert orientation were detected by restriction analysis by EcoRI and HindIII and transfected into CHO cells. The cells (transfected and non-transfected control cells) were harvested for 48 h following transfection. After sonication, the cells were concentrated by centrifugation and their protein profile was resolved in 12.5% reducing SDS-PAGE. The Western-blotting analysis showed that the mature proteins produced "in vitro" in CHO cells upon transfection with pcSAG1 has the expected molecular mass and recognized by specific polyclonal antibodies, whereas no T. gondii proteins were detected in non-transfected control cells.

p-522
CLONING AND EXPRESSION OF THE TYPE A BOTULINUM NEUROTOXIN BINDING DOMAIN IN BACILLUS SUBTILIS

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Neurotoxin of *Clostridium botulinum* is one of the most potent known toxins in nature. The toxin is expressed as single polypeptide chains with approximate molecular masses of 150 kDa. Fallowing proteolytic cleavage, the two chain linked by a disulfide bridge, results in an enzymatically active 50 KDa light chain, and a 100 KDa heavy chain. The binding domain is at C-terminal half and translocation domain is at the N-terminal half of the heavy chain. The use of recombinant binding domain has been proposed as a candidate vaccine. *Bacillus subtilis* has many attractive features to serve as an expression host for foreign protein. Some of these features include the non-pathogenic nature of B. subtilis, well-established safety record, ability to secrete extracellullar protein directly to medium, easy genetic manipulation and fast growth rate. In this study coding region of binding domain of *C.botulinum* toxin type A was amplified using a pair of primers containing HindIII and PstI restriction sites. The amplified 1300 bp fragment cloned in PWB980 vector and transformed into B.subtilis WB600 by electroporation. The transformants were screened by PCR or by restriction enzyme analysis and the presence of correct inserts was confirmed by DNA sequencing. Expression of the recombinant binding domain protein in B. subtilis was analyzed by SDS-PAGE and Western blotting.

p-523
P15 OVEREXPRESSION INHIBITS SMOTH MUSCLE CELLS PROLIFERATION AND INTIMAL HYPERPLASIA AFTER STENTING

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Arterial injury results in passage of smooth muscle cells (SMC) from G0 to G1 phase of cell cycle. Progression through the cell cycle is regulated by cyclin-dependent kinases (CDK) and requires inactivation of suppressor genes including p53, p27, p21, p16 and p15 that inhibit the kinase activity of the cyclin/CDK complexes. P15, a member of INK4 family, binds to CDK4 and prevents its association to cyclin D. The aim of this study was to investigate the effects of p15 overexpression on SMC proliferation and in-stent hyperplasia. Methods: In-vitro, rabbit aortic SMC were transfected with p15 or beta-gal adenovirus and transgene expression and cell proliferation were assessed at 24 hrs. In-vivo, p15 was overexpressed by adenoviral mediated gene transfection in stented carotid

arteries and transgene expression was evaluated at 24 hrs. Cell proliferation was evaluated by BRDU staining at 7 days and histomorphometric analysis was performed at 10 weeks. Results: PCR showed P15 transgene in SMC and carotid arteries. DNA synthesis was inhibited by 60% in p15 transfected SMC as compared to beta-gal transfected cells. At 10 weeks, intimal cross-sectional area of p15 treated group (0.61 ± 0.19 mm^2) was significantly lower than the beta-gal treated (0.91 ± 0.23 mm^2) and non-transfected (0.90 ± 0.18 mm^2) groups. Cell proliferation of P15 treated group was also significantly lower than beta-gal treated and metal stent groups. Conclusion: Adenoviral mediated overexpression of p15 results in inhibition of SMC proliferation and inhibition of intimal hyperplasia after stenting. Gene transfer of p15 may serve as a beneficial strategy to inhibit in-stent restenosis.

p-524
HERBICIDE-TOLERANCE IN TRANSGENIC POTATO PLANTS EXPRESSING BAR-GUS CONSTRUCT

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Potato is one of the most agronomical important plants in the world. Agrobacterium-mediated transformation of internodal explants, derived from two economically important Solanum tuberosum genotypes, namely Agria and Marfona, hase been successfully employed to generate transgenic plants resistant to herbicides. Marker genes of bacterial origin induce resistance to either an antibiotic (nptII gene) or herbicide (bar gene), or they encode an enzymatic activity easily detectable in the transformed tissues (gus/uid gene). In this study, transformation of plants was carried out with the C58C1RifR strain of *Agrobacterium tumefaciens* harboring the disarmed Ti plasmid pGV2260 with the binary plasmid pCABIA3301 which contains the intron-containing gus reporter gene and the bar selectable marker gene under control of the (CaMV) 35S promoter. DNA was isolated from putative transformants and positive and negative control plants. The presence of the transferred bar gene was demonstrated by using standard PCR techniques and β-glucuronidase enzyme activity was detected histochemically.

p-525
PRODUCTION OF A TISSUE PLASMINOGEN ACTIVATOR VARIANT BY SITE-DIRECTED MUTAGENESIS

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Tissue plasminogen activator (t-PA) is a multidomain serine protease of the plasminogen family. It was discovered that t-PA is capable of fibrin-stimulated clot lysis and that it could be used as an agent in the treatment of acute thrombotic disorders. Due to its rapid clearance from the circulation, t-PA must be infused to achieve thrombolysis. Bolus administration might further improve the lytic rate by quickly exposing the target clot to a higher concentration of the enzyme, but single bolus administration of wild-type t-PA is not generally used in the clinic due to its rapid clearance. An undesirable consequence of systemic activation is bleeding that may be related to plasmin generation rather than fibrinogen depletion. One way to reduce systemic activation is to make t-PA even more fibrin-specific. Systematic mutagenesis was applied to t-PA with the hope of increasing the fibrin specificity of t-PA. Mutations in the protease domain to substitute a tetraalanine at positions 296-299 were found to have this property. Substitution of asparagines at the position 103 by threonine that exhibits an additional glycosylation site also produced a variant with desired clearance rate. However, this mutation still did not yield full in vitro or in vivo fibrinolytic activity when compared with the wild-type t-PA. Additional mutation at the position 117 (replacing glutamine with aspargine) that removes a glycosylation site can recover the loss of activity due to previous mutations. In the present study, we have done these three mutations on the wild-type t-PA by PCR mediated site directed mutagenesis.

p-526
SELECTION OF POLYPEPTIDE VEHICLES FOR DELIVERING THERAPEUTICAL AGENTS ACROSS THE GI EPITHELIAL USING PHAGE DISPLAY TECHNOLOGY

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Intestinal barrier function regulates transport and host defense mechanisms at the mucosal interface. Transcellular and paracellular fluxes are controlled by membrane pumps, ion channels and tight junctions, adapting permeability to physiological needs. One of the major factors which affect the oral bioavailability of therapeutic agents is absorption through GI epithelial cell membrane. The development of delivery systems that transport therapeutic agents across the intestinal barrier has been considered challenging. Phage display technology provides a rapid means for discovery of novel peptides and proteins from genetically engineered variants. In this study, we utilized in vivo phage display (IVPD) in order to study the sequences responsible for transmucosal transport (TMT). We hypothesized that the introduction of a phage display library into the intestine would facilitate the identification of sequences that could induce TMT. Biopanning protocol was performed using a 7-mer random amino acid phage display library. Phage library were applied by gavage to rats and its translocation assessed by phage recovery from the spleen and blood. Following isolation, about 80 phages were sequenced and the frequencies of occurrence for amino acids were calculated. There are, however, few residues types which have been under represented which could be due to some specific GI selection mechanism and/or their effects on the amplification rate of phage particles bearing those residues. Investigation of the displayed peptides by the examined phages revealed the presence of some sequence similarities among them. In

conclusion our results support both specific and non-specific mechanisms for the GI transport of peptide displaying phage particles.

p-527
APPLICATION OF HUMAN BETA-GLOBIN INTRON-1 TO ENHANCE THE EXPRESSION OF HUMAN COAGULATION FACTOR IX IN MAMMALIAN CELLS

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Hemophilia B an X-linked recessive bleeding disorder affecting about 1 in every 30,000 males is caused by the functional deficiency of coagulation factor IX (FIX). Replacement therapy is a current treatment for this disease done by infusion of either human plasma derived FIX or its recombinant form. In order to produce a recombinant form of biologically active human FIX (hFIX), a mammalian expression system is necessary for proper post-translational modifications. A number of recent works show the transcription and pre-mRNA splicing are tightly coupled with gene expression events in eukaryotic cells that suggest for possible intronic functions. Bioinformatics analysis has also provided evidences for the presence of various regulatory and structural elements in the intronic sequences of the eukaryotic genes. Export of transcription product of nuclear gene like beta-globin is highly dependent on the presence of introns. Therefore beta-globin introns are suitable candidates for the expression of heterologous gene in mammalian hosts. In the present work the intron-1 of human beta-globin was inserted in its corresponding location in hFIX cDNA between the 1st and 2nd exons in a CMV promoter-regulated expression vector. After transfection of Chinese hamster ovary cells a number of colonies of tranfested cells were isolated and subjected for expression analysis based on biological activity experiments such as clotting test and ELISA. The outcome of this work together with the results which will be obtained from a parallel work on the application of intron-2 can provide tools and methods to achieve an efficient expression of hFIX, which can also be useful for overproduction of other eukaryotic proteins.

p-528
AMINO ACIDS SUPPLEMENTATION BASED ON STOICHIOMETRIC MODEL PREDICTION TO ENHANCE PRODCTION OF HUMAN RECOMBINANT INTERLEUKIN-2 IN E.COLI BL21

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The expression of recombinant proteins can lead to the depletion of amino acid pools of the host. Therefore, the provision of the most needed amino acids can improve the yield of production. In this work, a stoichiometric model was used to determine the role of various amino acids in the production of human interleukin-2 and to estimate their necessary amounts. According to the calculations of the model, Glutamine (0.32 g/l), Aspartic acid (0.22 g/l), Glycine (0.11 g/l) and Leucine (0.12 g/l) were identified as the most necessary amino acids which can improve the yield of productivity. Among these amino acids, the first three are used in the synthesis of DNA and RNA, and the last one (leucine) is the most common amino acid in the structure of human interleukin-2. The cell concentration of the samples which were supplied with glutamine and leucine were 50 and 36 percent higher than the standard sample, respectively. The sample containing Glycine had the most amount of total protein, which was 17 percent higher than the standard sample. Glutamine and Glycine had the most promising effects on productivity as shown by SDS-PAGE gel electrophoresis.

p-529
CLONING AND SEQUENCE ANALYSIS OF A NOVEL HALOALKALINE ZN-METALLOPROTEASE GENE FROM SALINIVIBRIO PROTEOLYTICUS

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In this investigation, for the first time nucleotide sequence of an extracellular protease gene from the moderately halophilic bacterium *Salinivibrio proteolyticus* was identified and reported to the GenBank. We generated a genomic DNA library from wild-type bacterium in E. coli and protease gene was screened by using agar plate-based assay. Among 10,000 transformants only one clone harboring plasmid pBluescript and 3.6 kb inserted fragment (pBlueSVP2) with positive caseinolytic activity was obtained. Nucleotide sequence analysis of the selected clone revealed a single open reading frame (ORF) of 1,833 bp encoding 611 amino acids. Analysis of the sequence upstream of the initiation codon allowed us to identify a presumable promoter region, TTGTTA for the -35 and TAAAAT for the -10 sequences, respectively. An inverted repeat sequence for the transcription termination exists at 16 bp downstream from the end of the ORF. A potential signal peptide of 22 amino acids was predicted at the N-terminus of the putative SVP2 protein using the Signal-P program. Furthermore, homology search of the deduced amino acid sequence with present proteases in the database showed a moderate homology (less than 70%) between this protease and the known zinc-metalloproteases including vibriolysin from Vibrio vulnificus (64%) and elastase of Pseudomonas aeruginosa (61%). In summary, analysis of the deduced sequence revealed that the isolated zn-metalloprotease from the Salinivibrio proteolyticus is transcribed and expressed as a preproprotein and converted to a mature form during secretion.

p-530
CELL TROPISM AND DIFFERENT PATTERN (SEVEN AMINO ACID SUBSTITUTION) IN 1C AND 1D PROTEINS OF FMDV TYPE-A VIRUS

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The initial step of infectious process of FMD virus is attachment to specific cell surface molecules. The G-H loop contains a highly conserved sequence of Arginine-Glycine-Aspartic acid (RGD) which has been implicated in the receptor binding. We analyzed VP1 gene sequence of two Iranian FMDV type A isolates. These isolates showed different cell tropism in BA, BHK-21 and CHO cell lines. The primary sequence alignment with other sequences from GenBank showed Seven aminoacids substitution I→V at two position 215, 203 of 1C protein; I→T at the position 48 , T→A at the position 93; rare substitution H→R at the position 142, R→K at the position 153 and V→A at the position 154 of 1D protein . Because of seven amino acid substitution in G-H loop and flanking region of 1D protein, it might cause a change in three dimensional structure of 1D receptor binding site and might have effective role in virus attachment in initiation of cell infection and virus not be able grow on BHK-21 suspension cell culture and CHO cell line.

p-531
MOLECULAR CHARACTERIZATION OF METALLOPROTEASE GENE IN DERMATOPHYTE

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Epidermophyton floccosum is one of the anthropophilic dermatophyte fungi which invade the skin of human. Several properties of this fungus have been investigated so far however a few studies were carried out in the field of molecular biology of this fungus. Metalloprotease is an extracellular keratinase which plays an important role in fungi pathogenicity and acts as a superantigen in stimulating the immune system. PCR preformed by designing one pair of 20 nt primers and using isolated genomic DNA of E. floccosum and the obtained fragments were then sequenced. At the present time, 690 nucleotides from this new gene which encodes a polypeptide have been sequenced. Nucleotide sequence comparison of this new gene which will hereafter is referred as EfMP in Genbank (NCBI, NIH), for both the partial DNA and its deduced polypeptide, revealed significant homology with members of the eukaryotic metalloprotease family. The amino acid sequence of the encoded protein was about 90% identical to the sequence of metalloprotease from other fungi.

p-532
CONSTRUCTION OF RECOMBINANT BIOPLASTIC PRODUCING GENES (PHB OPERON) AND E-LYSIS GENE FROM BACTERIOPHAGE PHI X174 IN E. COLI

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Polyhydroxybutyrate (PHB) is a biodegradable polymer produced by numerous gram positive and gram negative bacteria and accumulated as intracellular carbon and energy storage material under limited conditions. Since PHB was initially found by Lemoigen in 1926 there have been many investigations to replace it with petrochemical-based plastics. It has recently attracted much attention as a candidate for biodegradable thermoplastics because of increasing concern about environment pollution. *Ralstonia eutropha* has been studied extensively because of its ability to accumulate PHB to more than 80% (w/w) of the cellular dry weight. In this bacteria PHB is synthesized by acetyl CoA in three steps by function of three enzymes, β-ketothiolase, NADPH-dependent acetylcoA reductase, and PHA synthase which are encoded by phbA, phbB, phbC, respectively. These genes are located in the same operon in this order: phbC (1770 bp), phbA (1182 bp), and phbB (741 bp). In this project *Ralstonia eutropha* was obtained from Iranian Industrial Research Center. Bacterial genome was extracted and 16s rDNA was amplified by PCR with universal primers. Afterwards it was purified from gel and then sequenced by Bioneer Company. Sequencing results showed 99% identity with *Ralstonia eutropha* strain H16. After confirmation of bacteria, a pair of primer with ECORI restriction site was designed for the beginning and the end of phb operon. Forward primer was designed from 300 nucleotides upstream of the first gene, so it contained the bacterial promoter. Forward and reverse primers were also designed for E-lysis gene from bacteriophage phiX174. They contained NdeI and BamH1 restriction sites, respectively. In continution these genes will be amplified by PCR. E-lysis will be setteled under the control of thermal-inducible promoter (PL/CI) in the plasmid pZGY4. phb operon will be cloned in the same plasmid under the control of its natural promoter. After the maximum production of PHB in stationary phase the tempreture will be changed from 37 to 42 and gene E will be expressed, bacteria will be lysed and PHB can be extracted easily.

p-533
THE STUDY ON POSSIBILITY OF SOMATIC EMBRYOGENESIS IN POLIANTHES TUBEROSA

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Polianthes tuberosa (Amarilidaceae family) is an ornamental monocot and one of the most important cut flower. Colours of all the existing cultivars are white. Since this flower has not relative species introducing new variety is possible by in vitro mutation breeding and transformation. The studying on

regeneration (organogenesis or embryogenesis) as first step for creation of genetic variation is necessary. Vegetative explants (peduncle, leaf, node and internode) and different concentration of auxin (IAA, NAA, 2, 4-D) with or without BAP for induction of embryogenic callus were considered. Peduncle was the best explant for embryogenic callus induction. The highest embryogenic callus from peduncle explants was obtained on MS medium supplemented with 2 mgl^{-1} IAA and 0.5 mgl^{-1} BAP. Leaf explants were showed lower rate of embryogenesis in comparsion with peduncle. The highest embryogenic callus induction was obtained on MS medium supplemented with 0.5 mgl^{-1} IAA and 2 mgl^{-1} BAP. The node and internode explants were not suitable for callus induction.

p-534
TRANSFER OF A RECOMBINANT MURINE GLUCOCEREBROSIDASE GENE TO HELA CELLS BY LENTIVIRUS VECTOR

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Gene therapy has become one of the most intensively strategies for current clinical researches. Gene therapy offers new treatment for genetic deficiency. Recombinant gene technology was applied for transfer the correct gene to different cells in last decade. Deficiency of glucocerebrosidase (Gba) enzyme caused Gaucher disease and is attractive candidate for gene therapy. While there has been a great progress in gene therapy research, there is long way for being complete. The aim of this project is successfull transfection of Gba and mutated fragment to human embryonic kidney cell line and infection of HeLa cell by lentivirus. RNA extracted and glucocerebrosidase cDNA was amplified by PCR with specific primers. cDNA was cloned in non expressing vector. In frame mutation was made in cloned gene and sequenced. The Gba gene and mutated fragment was sub cloned in lentiviral vector derived by HIV-1. Recombinant lentivirus was produced by transfection of recombinant and packaging vectors into human embryonic kidney cell line and. The virus was exposed to HeLa cells and the glucocerebrosidase gene expressed. The cloned Gba cDNA in expression vector were correctly checked by restriction enzymes. The inframe mutation fragment and main glucocerebrosidase gene completely were sequenced. Sub cloning of glucocerebrosidase and mutated fragment in lentivral vector confirmed by restriction enzymes. The transfected HEK cells were checked by reporter gene. The infection of HeLa cells confirmed by fluorescent assay and a suitable titer of virus was produced. The part of gene therapy process was successfully achieved in this project. Gene therapy using lentivirus for gene transfer and long time gene expression will be applied for much native diseases in the future.

p-535
CLONING, SEQUENCING AND CHARACTERIZATION OF L-ASPARAGINASE FROM SHIGELLA FELEXNERI 2264

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Asparagine is one of the amino acid required for protein synthesis by cell. Tumor cells require huge amounts of asparagines to keep up with their rapid, malignant growth. L-asparaginase is an enzyme that destroys asparagine exteracellularly. The L-asparaginase of *Erwinia chrysanthemi*, *Erwinia crotovora* and E.coli (and some of Entrobacteriacae) has been employed for many years as an effective drug in treatment of acute lymphoblastic leukaemia. At the first step of this research S*higella flexneri* 2264 DNA was isolated according to a standard procedure. In the next step, we decided to design the specific primer of this gene by a software and information obtained from NCBI data base. PCR was performed with high-fidelity pfu DNA polymerase. Primer-extended PCR product was introduced into T-vector by T4 DNA ligase. Then, the cloning reaction was transformed into E.coli, DH5α and XL1Blue competent cells via Heat-shock transformation procedure. After overnight culturing, successful transformations were identified as white colonies. The plasmid was isolated through large scale plasmid isolation method. The isolated plasmid was verified by DNA sequencing. Then, we compared these sequences with other control sequences from Gene Bank.

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FULL-LENGTH CLONING AND EXPRESSION ANALYSIS OF ASR2 A SALT STRESS-INDUCIBLE GENE FROM *AELUROPUS LAGOPOIDES*

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Salinity is one of the most important factors that affect soils and limit plants production. About 6% of agricultural soils are affected with salinity. Salt tolerance is an important trait that varies between different plants. *Aeluropus lagopoides* (L) trin as a stoloniferous prennial grass, from poacea family, is a candidate to elucidate the physiological and molecular aspect of acclimation to adverse environmental condition. Previously, asr2 with CX779719 accession number was isolated from salt-treated *Aeluropus lagopoides* using cDNA-AFLP. Bioinformatics analysis showed that it encodes a probable DNA-binding protein. Entire length of asr2 obtaind using methods such as inverse-PCR and 3' and 5' Rapid Amplification of cDNA Ends (3' and 5' RACE) PCR. For analysis of gene expression pattern under different

environmental stresses semi quantitative PCR has been done by multiplex PCR method. Result suggests that a distinct physiology of asr2 in Aeluropus lagopoides salt tolerance.

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EXTRACTION AND PURIFICATION OF A RECOMBINANT TRIVALENT PERTUSSIS TOXIN-DIPHTHERIA TOXIN-TETANUS TOXIN FUSION PROTEIN

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Pertussis toxoid, diphtheria toxoid, and tetanus toxoid are key components of DTP trivalent vaccine. The antigens are derived from three different bacteria and the efficacy of the vaccines is well documented. In this study, a fusion protein composed of the immunoprotective S1 fragment of pertussis toxin, the full-length nontoxic diphtheria toxin, and fragment C of tetanus toxin was purified from a genetic engineered E.coli. To produce a soluble form of fusion protein, E.coli Rosetta-gami2 cells, harboring pCoPDT plasmid were grown in SB broth at 37^{o} C and expression was induced at 22^{o}C. Cells were harvested and then disrupted by sonication. The Ni-NTA chromatography using native buffers was used for primary purification of recombinant protein. Further attempts for more purification of recombinant protein using each DEAE-sepharose, hydroxy apatite, hydrophobic interaction, and gel filtration chromatoghraphies were unsuccessful. Proteins partially purified by IMAC were analysed by polyacrylamide gel electrophoresis using different sample buffers. The results revealed that rPDT protein was accompanied by a complex of contaminant proteins via disulfide bonds and hydrophobic reactions. The immunoblot analysis using either monoclonal or polyclonal antibody was not able to detect hsp60 in proteins complex. Electro-elution of partially purified recombinant protein yielded homogenous pure protein. The results of SDS-PAGE using reducing sample buffer showed a clear band with molecular weight of 161 kDa. Western immunoblotting showed that the purified PDT protein was recognized by the anti-S1, anti-TT, anti-DT, and anti-HA antibodies. These results indicate that the purified protein was the PDT fusion protein.

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ISOLATION OF A NOVEL MUTANT STRAIN OF SACCHAROMYCES CEREVISIAE BY AN EMS MUTAGENESIS APPROACH AS A HIGH BIO-ETHANOL PRODUCER

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In order to produce bio-ethanol more than the normal level, a commercial *Saccharomyces cerevisiae* type was subjected to mutagenesis by ethyl-methane sulfonate (EMS). After adding EMS to shaking yeast suspension, viability of yeast cells was measured by diluted sample inoculation to solid yeast-extract peptone glucose (YEPG) medium in each 15 minutes interval. At 45-minute point, the viability of yeast cells was estimated to be 30%. The mutant cells were recovered from broth YEPG after incubation at 30˚C for 18 h. After this period, distinct volume of mutant yeast cells were inoculated to solid aerobic low peptone (ALP) medium containing 2-12% (v/v) ethanol. All plates were incubated to 30˚C for several days in order to colonies formation. Mutant strains that tolerated high concentrations of ethanol used for bio-ethanol production in microfuge tubes, containing fermentation medium. Formation of bio-ethanol in small tubes was detected by distillation-colorimetric method. In addition, trehalose content and invertase activity were determined in each mutant strain. Among many isolated mutant strains, there were six isolated colonies that were grown on ALP medium supplemented by 10% (v/v) ethanol and one of them produced 17.3% bio-ethanol more than wild type. This mutant strain of yeast has a specific pattern of genes expression related to bio-ethanol production.

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SCP AND PCR AMPLIFICATION OF 18S rRNA OF *DUNALIELLA SALINA* ISOLATED FROM MAHARLU SALT LAKE

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Molecular markers are used as tools for estimating of the phylogenetic relationships of different kinds of organisms. Ribosomal RNA genes are among the most widely used genetic markers for phylogenetic analyses. Small subunit nuclear rDNA (18S) have been widely used for genetic identification of many organisms. Rapid population growth throughout the world is increasing the demand for protein based foods. Only a few studies have dealt with the feasibility of using single cell protein (SCP) from microalgae. Dunaliella is the only eukaryotic alga which can accumulate maximum amount of secondary β-carotene in its interthylakoid spaces. Dunaliella salina was isolated from water samples collected from Maharlu salt lake, Shiraz, Iran. The protein was assayed as described by Kochert and the protein content was calculated as the percentage of dry weight. SDS-PAGE was performed simultaneously in order to confirm that the amounts of proteins in the samples were equal. Total genomic DNA were isolated and used for PCR amplification of the 18S rRNA gene. Sequences were amplified using the universal eukaryotic primers. The molecular weight of the PCR amplified product was calculated and confirmed using gel documentation system. The green algae yielded a protein content of about 26%. The result of PCR blasted with other sequenced microalgae in NCBI showed homology to the 18S small subunit rRNA of other microalgae. The evidences suggest that Dunaliella, with high content of good quality protein, is a potentially valuable food source.

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SCP AND PCR AMPLIFICATION OF 18S rRNA IN SCENEDESMUS

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Scenedesmus is a unicellular green alga which belongs to the order of chlorococcals. Scenedesmus is a small, nonmotile colonial green alga consisting of cells aligned in a flat plate. Small subunit nuclear rDNA (18S) have been widely used for genetic identification of many organisms. Algae are receiving wide attention as a source of biomass protein (BMP) for use as food. A few studies have dealt with the feasibility of using single cell protein (SCP) from microalgae. *Scenedesmus obliquus* was isolated during a screening program from soil samples collected from paddy-fields of Fars province, south of Iran. After colonization, pure cultures of living specimens were prepared using subculturing with agar plate method in BG-11 medium. The protein was assayed as described by Kochert and the protein content was calculated as the percentage of dry weight. SDS-PAGE was performed simultaneously in order to confirm that the amounts of proteins in the samples were equal. Total genomic DNA were isolated and used for PCR amplification of the 18S rRNA gene. Sequences were amplified using the universal eukaryotic primers. The molecular weight of the PCR amplified product was calculated and confirmed using gel documentation system. The green algae yielded a protein content of about 37%. The result of PCR blasted with other sequenced microalgae in NCBI showed homology to the 18 S small subunit of rRNA of other microalgae. Scenedesmus might be a good lipid and protein source for human consumption. We should therefore look forward to extensive use of SCP products in future.

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THE SIGNIFICANCE OF HETEROCHROMATIN POLYMORPHISM AND HUMAN LEUKEMIAS

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Introduction: Polymorphisms of the size of heterochromatin regions of chromosomes have been well documented in human genome and it consists of DNA sequences that are not transcribed. This study represents the first report of polymorphism of heterochromatin regions of chromosomes investigated in Iranian populations. Hence, the aim of this study was to evaluate the heterochromatin polymorphism associated with chromosomes in leukemic patients and healthy normal controls. Material and Methods: This randomized collected study was conducted on 35 consecutive leukemic patients and 34 healthy individuals in Modaress and Taleghani hospitals, Tehran, Iran from 2004 to 2006. Applying Barium Hydroxide saline Giemsa (BSC) method with some modification, the variant heterochromatin polymorphism of chromosomes 1, 9 and 16 on bone marrow and lymphocyte cultures were evaluated. Results: Constitutive heterochromatin polymorphism of chromosomes 1 and 9 in leukemic patients showed highly statistical significance when compared with chromosomes of healthy controls ($P = 0.0005$ and $p = 0.006$, respectively). The differences were not significant for chromosome 16, it was 11.4% in leukemic patients and 0% in the control group ($P=0.05$). The frequency of partial and complete inversion did not also showed significant difference between the leukemic patients and the control group. Conclusion: The constitutive heterochromatin polymorphism blocks could possibly serve as a marker to detect and characterize the chromosome in leukemias patients.

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STUDY OF CHOLESTEROL OXIDASE ACTIVE SITE IN RHODOCOCCUS SP BY SITE-DIRECTED MUTAGENESIS

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Cholesterol oxidase is a monomeric flavoenzyme which catalyzes the oxidation and isomerization of cholesterol to cholest-4-en-3-one. Site-directed mutagenesis was used to identify the key amino acid residues responsible for catalytic reaction of the cholesterol oxidase from Rhodococcus sp. The structure of this enzyme in other microorganism suggests that Glu361, located at the active site cavity, may act as the base for both the oxidation and the isomerization steps of the catalytic reaction. Some substitutions at Glu361 were produced by PCR- based site-directed mutagenesis. Three mutant enzymes were constructed and following amino acid substitutions were identified: E361N (Asn), E361Q (Gln), E361D (Asp) .The wild –type and mutant enzymes were purified and characterized. They are compared by their kinetic properties. Results will be presented.

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DETECTION OF VACA SUBTYPES OF HELICOBACTER PYLORI STRAINS IN SHAHREKORD

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Helicobacter pylori is one of the important pathogens responsible for chronic gastritis, peptic ulcer and gastric

cancer. H. pylori is a medically important bacterium that infects about half of the world population. Vacuolating cytotoxin gene A (vacA) is present in nearly all H. pylori strains reported from all around the world and contains at least two variable parts. The s region (which codes the signal peptides) exists as s1 and s2, and the m region (the middle region) occurs as m1 and m2 allelic type. The present study aimed to detect vacA subtypes of H. pylori strains in Shahrekord. Biopsy samples were collected from 95 patients who referred to endoscopy department of HAJAR hospital in Shahrekord because of dyspeptic symptoms. At first we used RUT method for H. pylori detection. Then DNA was extracted directly from biopsy specimens that were positive by RUT test and PCR-amplification preformed for the vacA gene. Amplified fragments of 259 and 285 bp were expected from genotype s1 and s2, respectively. The middle region of the vacA gene was analyzed with primers which amplified 570 bp fragments for m1 and 645 bp fragments for m2. The present study revealed that combination of s1m2 was predominant. s1m2 was more prevalent in the strains obtained from ulcer patients and s1m1 more prevalent in the strains from NUD (Nonulceration disease) cases.

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ELECTROCHEMICAL DETECTION OF UGT1A9 -275 (T/A) SINGLE NUCLEOTIDE POLYMORPHISM USING PNA-GOLD BIOSENSOR

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UGT1A9 gene is the key uridine glucuronidase transferase enzyme responsible for glucuronidation of most drugs and is predominantly active in the liver, kidney and intestine. In human liver microsomes, UGT1A9 expression correlates with specific single nucleotide polymorphisms (SNPs) in the gene promoter region including substitution at position -275(T/A) that influence the drug metabolism and consrquently drug serum level and drug's therapeutic and side effects. Sequence analysis has been recently achieved using peptide nucleic acid (PNA) recognition layers. This study aims to specifically detect the nucleotide substituation at position -275 in promoter region of UGT1A9 using PNA-based biosensors. Following modification of the gold electrode (AuE) surface for electrochemical experiments, the PNA probe immobilized onto the electrode using its thiol group. Then electrochemical transduction of hybridization between the probe and complementary/non-complementary DNA sequences was performed. The complementary and non-complementary DNA targets had the same sequences except one nucleotide switching between A and T. The detection of hybridization was accomplished via the reduction of methylene blue (MB) indicator. We observed that the immobilized PNA probe can attract DNA target sequence onto the AuE surface so strongly that it couldn't be washed away easily from the probe. This strong binding was resulted from the full matched hybridization between PNA probe and DNA target. PNA-DNA hybridization was significant as detected using electrochemical approach. Our experiments showed that electrochemical signal was reduced remarkably in the presence of non-complementary target DNA. The reduction in electrochemical signal is attributed to the single base mismatch between the PNA probe and DNA target. This observation led to the effective discrimination against target DNAs with single point mutation. So we can conclude that the constructed PNA probe with modified Au electrode can effectively detect point mutation in UGT1A9 promoter region (-275) by MB as an electroactive reporter.

p-545
GENERATION OF RECOMBINANT ADENOASSOCIATED VIRUS (AAV) PARTICLES CONTAINING LYSOZYME SIGNAL, THE SINGLE DOMAIN ANTIBODY AND GFP

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AAV are small, non pathogenic paroviruses that require co-infection with a helper virus such as adenovirus or herpes virus for production. The ability of AAV vectors to infect dividing and non-dividing cells and establishing long term transgene expression and the lack of pathogenecity have made it attractive for use in gene delivery. Cloning of lysozyme signal –VHH-GFP (signal peptide – single domain camel antibody and green fluorescent protein) was performed described briefly as follows. The GFP was amplified from pEGFP-c1 by using primer sets and was cloned into Hind III and BglII sites of AAV-MCS viral vector. The new construct obtained in this way was named as AAV-MCS-GFP. Lysozyme signal –VHH encoding sequence was produced by splicing overlapping extension PCR (between synthetic lysozyme signal and VHH) and was cloned into puc18 vector using the restriction enzyme sites BamHI and Hind III. The cloned gene was then digested out, gel purified and ligated upstream of GFP into the AAV-MCS-GFP. The product construct was named AAV-MCS-Lyz-VHH-GFP. The viral vector containing the desired gene was used in this research. AAV-293 was cultured in DMEM containing %10 FCS. Co-infection was performed in 70% confluence with AAV-MCS-Lyz-VHH-GFP, AAV-helper and AAV-RC with calcium phosphate procedure. After 72 h cells were harvested and freeze thawing was performed at 37^{O} C and dried with ethanol 4 times. Centrifuged at 10000 X g and supernatant was used for injection. The viral production was detected by fluorescent microscopy, fluorescent spectroscopy and ELISA.

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NUCLEOTIDE SEQUENCE OF cDNA CODING FOR GOAT PROCHYMOSIN

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Chymosin (rennet) is the major proteolytic enzyme in stomach secretion of all nursing mammals and is used in cheese manufacturing as a milk coagulant because it cleaves k-casein in a specific manner. World shortage of calf rennet due to

increased demand for cheese production has intensified the search for other coagulants such as aspartic acid proteinases of microbial and plant origins as well as recombinant calf chymosin produced by different microorganisms. The purpose of this research is to produce a recombinant chymosin from goat. In order to determine the nucleotide sequence of the goat prochymosin, total RNA was isolated from the abomasums of 7-10 days old kids (*Capra Hircus*) and the single strand cDNA was synthesized using M-MuLV reverse transcriptase and primer ReEcoR. The double strand DNA then was synthesized using pfu and primers ReEcoR and ReOutR. The amplicon was cloned into the plasmid pTZ57R. The nucleotide and deduced amino acid sequence analysis revealed that, like the calf pro-chymosin, the cDNA encodes a 42 amino acid peptide pro-enzyme region and 325 amino acids for mature enzyme. The deduced caprine pro-chymosin sequence contains three disulfide bond linking cys 45 to cys 50, cys 206 to cys 210, and cys 250 to cys 283. In addition, two aspartic residues, Asp 32 and Asp 215 which function as catalytic residues in aspartic proteinase are present in goat chymosin.

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DESIGN AND PRODUCTION OF EXPRESSION VECTORS FOR ASPARAGINASE II IN E.COLI

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Asparaginase therapy is a well established treatment in leukemia and some other blood related lymphoma. Asparaginase II from E. coli has been approved by FDA and other agencies as the main form of the enzyme for therapeutic applications. However, one of the main setbacks for wider prescription of this therapy is high cost due to difficulties concerning expression and purification of this enzyme. Here, we sought to examine new expression vectors to increase yield and location of expression of asparaginase II in E. coli expression strain BL21. At first (ansB) was extracted from PGEM vector by the use of this 2 enzymes (ECO R1 and NDE1) then ligated in PET28 vector and competented DH5α cells were transformed by this vector. After this process the digestion of extracted plasmid with the restriction enzymes that was mentioned before revealed that insertion of (ansB) was complete. The extracted plasmid was used to transform competent BL21 cells to reach the highest amount of the enzyme expression. The transformed BL21 cells were cultured in LB broth with treatment of IPTG to enhance the expression. The sampling process accrued each 2 hours (5 times) and the last one after over night incubation. Production of the enzyme was confirmed by the SDS-PAGE gel method. Furthermore, we tested different simple (M9) and complex (LB) media to investigate the effect of carbon source on expression level of asparaginase II in E.coli.

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MOLECULAR PHYLOGENY ANALYSIS OF A NEW STRAIN FROM KLEBSIELLA PNEUMONIA SP. BASED ON 16S rRNA AND XYLOSE ISOMERASE (XLYAS) GENE SEQUENCE

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A new gram positive, aerobic bacilli which have an optimum temperature of 40°C isolated from cane factory and have been subjected to a molecular phylogeny analysis. The genes coding for 16S rRNA and XlyAs from Klebsiella pneumoniae TM100 was isolated, sequenced, and used for phylogeny analysis. Our group sampling included 19 known bacteria XlyAs and 16S ribosomal DNA (rDNA) obtained from the databases. Phylogeny studies of the K. pneumoniae TM100 with 19 other bacteria showed that this group of organisms could be divided in two clusters according to the XlyAs sequence. One cluster was C+G rich DNA and the other with a lower C+G content. Furthermore within the second cluster the K. pneumoniae TM100 was most closely related to the K. pneumoniae and E.coli (99 and 79% ihomology, respectively). In addition, comparative sequence analysis of 16S rRNA revealed incongruity between the XlyAs and 16S rDNA phylogenetic trees, which suggests that there has been horizontal transfer of XlyAs genes.

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ISOLATION AND CHARACTERIZATION OF CRUDE OIL

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Crude oil continues to be used as the principal source of energy and hence an important global environmental pollutant. Microbial decontamination appears to be the most environmentally friendly method of removal of oil pollutant. The purpose of the present study was to investigate a possible method of biodegradation to enhance the decontamination rate of crude oil. Enrichment of degrading microbes and inoculation them into the contaminated site can be used for removing hydrocarbon pollutant from the environment. The bacillus.sps was isolated from a contaminated area close to the storage and distribution center of oil products. Forty bacillus monocultures were isolated and their capability to grow on crude oil and to produce surfactant and hydrolytic enzyme was determined. We set up experiment using 1%-3% (v/v) crude oil in MSM medium. The results indicated the ability of the bacillus to produce sufficient biosurfactant. The maximal increase in OD 600 nm, protein concentration (measured by Bradford assay) and total viable counts were concomitant with decrease in pH of culture media in sixth day of the experimental period. Typical generation time varies between 18 to 26 hours. There was a positive correlation between bacterial growth and decrease of surface tension. The best isolated bacillus decreased surface tension from 65 (mN/M) to 37 (mN/M) after 48 hours. Quantitative analysis using gas chromatography carried out to correlate the bacterial growth and reduction of crude oil. Further understanding of the metabolic process of these organisms will increase the possibility of developing models and strategies for removing hydrocarbon pollutants from oil contaminated environments.

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THE RELATIONSHIP BETWEEN HAPLOTYPES & IVSI-6 MUTATION IN β-THALASSEMI

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Objective: β-thlassemia is a monogenic disorder of hemoglobin synthesis. Many individuals are suffering from the disease in the world and due to high burden of the disease, the control of thalassemia is very important. The disease is caused by more than 200 different mutations in β-globin gene with different severities. IVS1-6 mutation is a β++ thalassemia which usually causes thalassemia intermedia in homozygous form. The aim of this study was to find the relationship between IVS1-6 mutation and β-globin cluster haplotype among 18 β-thalassemia alleles in Iranian carriers of β-thalassemia. Materials & Methods: Genomic DNA was extracted from 5 ml of peripheral blood of Iranian carriers of β-thalassemia referred from Primary Health Care (PHC) centers. IVS1-6 mutation was tested by amplification refractory mutation system (ARMS) PCR. For haplotype analysis 3 different site in β –globin cluster (Gy-HindIII, 3'□ HincII, β-AvaII) were analysed using PCR-RFLP. Results & Discusion: Among carriers of β-thalasemia, 18 individuals had IVS1-6 mutation in β-globin gene. The haplotype analysis showed 80% had the haplotype VI (+ - -). For the remaining individuals no definite haplotype could bedetermined. This could be due to genetic recombination between these alleles at β-globin cluster in "hot spot" areas.

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MOLECULAR CLONING OF GP63 ANTIGEN OF LEISHMANIA MAJOR STRAIN MRHO/IR175/ER

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Background: Gp63 is the major surface glycoprotein of Leishmania that exhibits protease activity and has an important role in the biology of the parasite. The aim of this study was cloning and sequencing of Gp63 of L. major strain MRHO/IR175/ER. Methods: L. major promastigotes were grown at 24^{o} C in RPMI1640 supplemented with 10% FCS. L. major RNA extraction and cDNA synthesis were carried out using standard methods. Gp63 gene segment was amplified by specific primers and cloned into pTZ57R to construct pTZ57R/gp63 by T/A cloning vector. pTZ57R/gp63 was transformed into E.coli DH5-α and cultured on LB agar containing ampicillin, X-gal and IPTG. After 16 hours incubation, white colonies were chosen. The presence of gp63 into pTZ57R was confirmed by PCR on the plasmid pTZ57R/gp63 extracted from white colonies. Then, pTZ57R/gp63 was verified by direct sequencing of nucleotides. Results: PCR method confirmed a successful cloning of gp63 into pTZ57R. There were some differences between the sequence of nucleotides of gp63 of L. major strain MRHO/IR175/ER and other reports. Discussion: Today researchers attempt to find a suitable vaccine for leishmaniasis. Although some researchers have reported proper vaccines, these vaccines are not effective in other societies. The reasone might be because of differences between strains in different areas. Therefore, it is necessary to find the molecular information regarding leishmania strains causing diseases in our region. To our knowledge this is the first report about gp63 of L. major in Iran and it can be used in several researches for vaccine synthesis against leishmaniasis.

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A COMPARATIVE STUDY OF PCAMV 35S AND GBSSI PROMOTERS FOR HUMAN CALCITONIN GENE EXPRESSION IN TRANSGENIC POTATO PLANTS

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In recent years, the tools of genetic engineering have allowed development of transgenic plants that can express various recombinant biopharmaceutical compounds, including viral and bacterial antigens, antibodies, and various human and animal therapeutic proteins. Because of the lack of contamination with viral or bacterial materials, mammalian pathogens, low cost and industrial storage of recombinant protein in specific plant organ or organelle, host plants that produce recombinant protein are important in modern technology. Promoters of reserve proteins in seeds and in vegetative reproductive organs of plants are strong promoters and determine high level of synthesis (in specific organs and tissues) of not only corresponding storage protein but also the foreign products. Granule bound starch synthase I (GBSS I) was previously implicated as the enzyme for amylose synthesis in tuber storage starch and was found completely within the granule matrix. Calcitonin is a peptide hormone synthesized by the parafollicular cells of the thyroid. It is a 32 residue peptide hormone known to participate in calcium and phosphorus metabolism. Calcitonin also appears to be a valuable aid in the management of certain types of osteoporosis. In this project, to increase human Calcitonin expression, potato tuber organ specific promoter was used to construct GBSSI/hCT for *Agrobacterium tumefacience* LBA4404-mediated potato transformation. Transgenic plant was selected in selective culture contained kanamycin. The results showed that the GBSS I promoter have increased the yield of the expressed recombinant human Calcitonin hormone four-fold in comparison to pCaMV 35S (Cauliflower mosaic virus 35S promoter).

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CLONING AND TRANSFORMATION OF TISSUE PLASMINOGEN ACTIVATOR cDNA IN TOBACCO PLANTS

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Because of features such as high safety, low cost, post-translational modifications and high volume of production plants offer a promising alternative to microbial fermentation and animal cell cultures for production of recombinant proteins. In this report, recombinant cDNA of tissue plasminogen activator was transformed in tobacco plants. This recombinant protein was expressed under the control of CaMV35S promoter, and Nos terminator. The kozak sequence for high-expression sequence and KDEL signal for storage of recombinant protein within endoplasmic reticulum were linked at amino and carboxy-terminies of tPA gene, respectively. The construced cassette (pBItPA) was transferred to Agrobacterium, and the tPA gene was inserted into the plant genome by Agrobacterium-mediated transformation. Transgenic plants were selected on kanamycin (50-100mg/L), and maintained in perlite and then soil, and subsequent generation was obtained. The presence and expression of the transgene was confirmed in the transformants by Polymerase Chain Reaction (PCR), SDS-PAGE (PolyAcrylamide Gel Electrophoresis), and RT-PCR.

p-554
COMPARISON OF EXPRESSION O TWO DIFFERENT FORMS OF PROCHYMOSIN GENE IN TWO E.COLI BL21 RECOMBINANT STRAINS

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Chymosin is one of the enzymes with many applications in food industry. Its recombinant type has been designed and constructed in different forms. In this investigation, cDNAs synthesized from mRNA pools were extracted from the mucosa of the calf's fourth stomach. They were examined and a new type of exon-6less cDNA (alternative splicing form) was identified. The natural and exon-6-less forms of the mentioned gene were cloned into E.coli strain of BL21. In order to compare protein expression of above two strains, a set of experiments were designed. In all of these experiments, concentration of the inoculum was 5% v / v and aeration of 250 rpm was used. The effects of factors such as temperature, OD for induction of protein production, IPTG concentration and time after induction were evaluated. After sampling, the quantity and quality of protein were examined with SDS-PAGE and the following results were obtained. The comparison of quantities obtained from the gel scanner indicates that protein expression in the exon-6less form is noticeably higher than that in the natural form. The optimized conditions for the two mentioned strains are as follows: temperature of 37˚C, OD of 1.2 for induction of protein production, IPTG concentration of 0.5 mM and sampling at 4 hours after induction for the natural strain; a temperature of 37˚C, OD of 2.4 for induction of protein production, IPTG concentration of 1 mM and sampling at 4 hours after induction for exon-6less strain. A comparison of the above two optimized conditions indicated that the protein expression levels in this two conditions are 35.008% and 49.584% of the total cell protein for the natural and exon-6less strains , respectively.

p-555
INVESTIGATING THE SOLUBILIZATION OF INCLUSION BODIES (IB) CONTAINING RECOMBINANT INTERFERON- β-1B

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Since human interferon-β (IFN- β) is an antiviral glycoprotein which is used clinically in the treatment of multiple sclerosis (MS), its production is very important. With respect to the structure and biological properties, interferons are classified into two major groups: class I and class II. IFN- β belongs to class I and is produced by fibroblasts, leukocytes, lymphocytes, and the immune system. Currently, two kinds of recombinant IFN- β with biological activity and clinical usage are produced. The glycosylated form is expressed by animal cells or yeast (IFN- β-1a) and the nonglycosylated form can be obtained by expression in E.coli (IFN- β-1b). A synthetic gene encoding 166 residues of IFN- β-1b was cloned into E.coli resulting in 24% expression, and the recombinant IFN- β-1b was synthesized as an inclusion body (Ib). The molecular weight of r- IFN- β-1b was approximately 20 KD. In this project, we investigated the solubilization of Ib and the refolding of r- IFN- β-1b prior to purification. Solubility of Ib was examined by the following procedures: 1. Solubilizing the Ib by changing pH 2. Studying the effect of urea concentration on the Ib solubilization. 3. Studying the effect of incubation time on Ib solubilization. 4. Studying the effect of guanidine hydrochloride concentration on the Ib solubilization. 5. Studying the effect of Ib concentration on Ib solubilization. The SDS-PAGE analysis including the above examinations and estimation of Ib weight before and after solubilization (in order to calculate percentage of Ib solubilized) showed that solubilizing Ib in 8 M urea buffer, pH 10.5 at room temperature, for 3 hours was better than the other examined procedures. The solubilized Ib including r- IFN- β-1b refolded after over night dialysis against tris buffer, pH 10.5, at 4^{o} C. The refolded r- IFN- β-1b was then purified by affinity chromatography using a Blue – sepharose column. SDS-PAGE analysis of fractionated samples showed that purification was not absolute by this procedure, where purity was approximately 75%. Identity of the recombibnant protein was confirmed by western blot analysis. Biological activity of this product was assessed by application of the antiviral properties of interferons. Two peaks obtained from chromatographic analysis including the relatively purified r-IFN- β-1b were tested by the cytopatic effect reduction (CPE) method, using HeLa cell lines (sensitive to interferon) and vesicular stomatitis virus (VSV) as the lytic virus. We found that both peaks containing the relatively purified r- IFN- β-1b were biologically active.

p-556
THE FREQUENCY OF β – GLOBIN GENE MUTATIONS IN INDIVIDUALS REFERRED TO PND UNIT AT PASTEUR INSTITUTE OF IRAN

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Objective and Background: β-Thalassemia is a monogenic disorder caused by reduced or absence of β-globin chain synthesis in hemoglobin tetramer. The disorder is the most common hereditary disease in the world. It is caused by more than 180 different mutations in β-globin gene. The aim of this study was to find the frequency of different mutations at β-globin gene in β-thalassemia carriers referred to PND unit at Pasteur institute of Iran. Material and Methods: This study includes 234 β-Thalassemia carriers referred from primary health care centers during premarital screening program for β-Thalassemia prevention. Genomic DNA was extracted from 5 ml peripheral blood by standard methods. 30 common mutations in β-globin gene were screened by amplification refractory mutation system (ARMS) PCR method. When the mutation was not found, direct DNA sequencing of β-globin gene was performed by chain termination method. Results and Discussion: Among the 234 individuals, mutations distribution showed some differences in various regions in Iran. Some of mutations were distributed in almost all parts of the country like IVS II-I, IVS I-5, cd8/9, cd36/37 and some of them were seen in the special regions (e.g.: IVS 1-25del). The frequency of mutations was: IVSII-I (36%), IVSI-5 (10%), Fr 8/9 (9%), Fr36/37 (8%), IVSII-745 (8%), IVSI-110 (5%), IVSI-6 (4.5%), -88 (4%), C5 (3.5%), C22 (3%), IVSI-25 (3%), IVSI-I (2.5%), C44 (2.5%), +22 (2.5%), -30 (2.5%), C30 (2.2%), C 82/83 (1.7%), C22/24 (1.7%), C37/39 (1.5%), C39 (1.5%), C54 (1%), C15 (1%), -28 (1%). This survey shows that the distribution of mutations in the country is in agreement with previous studies. Finding prevalent mutations at β-globin gene in β-Thalassemia carriers provide invaluable help for rapid prenatal diagnosis of this disorder, providing that final decision should be made before 16th week of gestational age.

p-557
ANTIFUNGAL ACTIVITY OF CHITINASES ISOLATED FROM BACILLUS PUMILUS SUBSP. SG2 IN IRAN

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Chitinase enzyme has been vastly used as a pesticide and biological control agent against plant fungal and bacterial pathogens. Different chitinase genes have been isolated from various organisms that show low homology together. Previously, we showed its high chitinolytic activity. Bacterium *Bacillus pumilus* subsp. SG2 produces two chitinases called ChiS and ChiL and secretes them into the medium. In this study, two chitinase genes (chiS and chiL) of *Bacillus pumilus* (isolated in deserts of Iran) were identified and characterized.ChiS consists of Glyco18, Fn□□□ and CBD (chitin binding domain). chiS and chiL were cloned into Top10 strain of E.coli and after confirmation of protein production, their enzymatic activity were investigated. Antifungal activity of constructs including these chitinase genes was assessed on *Sclerotinia sclerotiorum* and Nigrospora sp and their inhibitory effect was confirmed.

p-558
CLONING AND CHARACTERIZATION OF TETANUS TOXIN FRAGMENT C

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Tetanus toxin (TeTx), a neurotoxin produced by *Clostridium tetani*, is a 150-kD protein, containing a 50-kD light-chain linked with a disulfide bond to a 100-kD heavy chain at amino-terminal. Fragment C, the 50-kD carboxy-terminal portion of the heavy chain, has ganglioside binding and protein binding activities. It is antigenically active, atoxic, and could stimulate the formation of antibodies neutralizing the lethal action of tetanus toxin in vivo. Recent report proposes that this fragment could be used as a vaccine against tetanus. Harvard strain of C.tetani which is the vaccinal strain for preparation tetanus vaccine in Razi institute was used for isolation of this gene. After culture on selective media in anaerobic condition, DNA was extracted by absorption of DNA on glass fibers (Template DNA purification, Roche, Germany). Specific primers were designed by oligo Software. The fragment C of tetanus toxin was amplified from *Clostridium tetani* DNA by PCR. The 1.4 kb fragment was cloned into pTZ57R/T cloning vector, and colonies were screened by blue /white selection. The plasmid containing fragment C was isolated, purified and used for sequencing. Fragment of Harvard strain was analyzed and characterized by insilico methods using DNAMAN software.

p-559
STUDY OF MOLECULAR MECHANISM OF SALT-RESISTANCE IN A MODERATELY HALOPHILIC BACTERIUM ISOLATED FROM URMIA SALT LAKE

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Moderately halophilic bacteria include a heterogeneous group of microorganisms which have benn adapted to survive in hypersaline environments. These microorganisms are capable of using several molecular mechanisms for osmoresistance. In the present study a moderately halophilic bacterium was isolated from Urmia salt lake, Northwest of Iran. The sequence of 16S rRNA gene was analyzed to classify this strain phylogenetically. It appeared to have the closest association with Halomonas ventosae. This was also confirmed by some phenotypic traits. Bacterial growth was monitored in LB media containing different amounts of NaCl (1-20% w/v). The optimal growth was observed at 7.5% NaCl.

To study the molecular aspects of salt resistance we focused on proteins that might be involved in response to salinity in this microorganism. Total cell lysates were extracted and then subjected to polyacrylamide gel electrophoresis. Analysis of protein profiles revealed a distinct peptide band. This specific band was obtained from proteomes of those samples that had been cultured in media containing more than 5% NaCl. It was then blotted on PVDF membrane and partially sequenced from the N-terminal. Furthermore, peptide finger printing using MALDI-TOF analysis revealed that the peptide has the most similarity with ribosomal protein S4 which belongs to *Chromohalobacter salexigens* DSM 3043. Subsequently, correspondent primers to the obtained amino acid sequence were synthesized. Analysis of the resultant PCR products gained more insight into the primary structure and function of this peptide.

p-560
SEX DETERMINATION IN NMRI MOUSE BASED ON SEX DETERMINING REGION OF Y CHROMOSOME BY PCR

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The history of ideas on how the sexes became divided spans at least three thousand years. Accurate and rapid sex determination of preimplantation embryos has great potential both in animal breeding and in human pathology. The gene SRY (sex determining region of Y), located at the distal region of the short arm of Y chromosome, is necessary for male sex determination in mammals. SRY initiates the cascade of steps necessary to form a testis from an undifferentiated gonad. In this study a set of specific primers were designed for mouse SRY and ZFX genes. Genomic DNA extracted from white blood cells of adult males and females mice using phenol/chloroform method. At first the specificity of the primers were tested on 15 DNA samples drived from 8 males and 7 females of NMRI mice, and sensitivity of PCR estimated <10pg using serial dilution of male DNA. ZFX primers used as an internal positive control for PCR amplification. The experiments reported here indicate that SRY can be successfully amplified from single mouse blastocyst. We conclude that SRY gene amplification can represent a good marker for embryo sex determination.

p-561
EXPRESSION OF ROTAVIRUS SPIKE PROTEIN (VP4) IN THE BACULOVIRUS EXPRESSION SYSTEM

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Rotaviruses are the major pathogens that cause life-threatening gastroenteritis in young children and animals world wide. Rotaviruses are members of family, Reoviridae. The mature rotavirion is an icosahedron consisting of three concentric shells of protein that surround a genome of eleven segments of double-stranded RNA. VP4 proteins function as spikes on rotavirus outer capsid and virus bind by them to its receptor. VP4 also induce production of neutralizing antibodies. But, immunogenicity of VP4 is unknown. We cloned simian rotavirus SA11 gene 4 cDNA into a cloning plasmid pDONRTM by bacteriophage λ BP recombinase and recombination factors. The resulted clone was called VP4-entry clone. In the second recombination reaction we inserted cloned gene into the linear DNA of the Baculovirus Autographa californica Nuclear Polyhedrosis Virus (AcNPV) adjacent to the strong polyhedron promoter by bacteriophage λ LR recombinase and recombination factors in vitro. The recombinant AcNPV DNA was transfected into the insect cell line [Spodoptera frugiperda (Sf9) cells] cultured in Grace Medium in room temperature. Expression of VP4 in the Sf9 cells, were confirmed first by immunofluoroscence (IF) test, using rabbit polyclonal anti-rotavirus and anti-rabbit FTIC conjugated antibodies. We also confirmed expression of VP4 in Sf9 cells by western blotting. Reactivity with anti-rotavirus antibody suggested that, VP4 expressed intracellularly, mediated native antigenic determinants. This work will be continued to purify and to study immunogenic properties of VP4 for recombinant vaccine production.

p-562
EVALUATION OF AMBIGUOUS THALASSEMIA CARRIERS REFERRED TO PND UNIT AT PASTEUR INSTITUTE OF IRAN

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Background and Objective: The thalassemias are a group of inherited anemic disorders of defects in globin chains synthesis. Typically, beta thalassemia carriers have low MCV and MCH and raised HbA2. Ambiguous carriers are designated as low indexes and normal HbA2. The main differential diagnosis includes iron deficiency anemia, alpha thalassemia and normal HbA2 beta thalassemia. The aim of this study was hematological family study and molecular characterization of alpha and beta globin genes in ambiguous carriers referred to PND unit at Pasteur institute of Iran. Materials and method: This study includes 488 ambiguous thalassemia carriers referred from primary health care (PHC) centers. After obtaining informed consent, the blood samples were collected in EDTA tubes. Genomic DNA was extracted using the salting out method. ARMS- PCR and DNA sequencing was performed for finding the mutations in beta globin gene. Multiplex Gap PCR was exploited for detection of alpha globin gene deletions. DNA sequencing for point

mutations in alpha globin genes was performed for appropriate cases. Results and discussion: Of 488 cases, 4 individuals had mutations in beta globin gene. The mutations were: Fr8/9, -30, +22 and large deletione in beta globin gene. The remaining were carriers of alpha thalassemia or normal. Normal HbA2 in beta thalassemia is not common and for finding the causes of this phenomenon the type of mutation in beta globin gene (beta + +, beta silent) in combination with iron deficiency anemia or alpha thalassemia should be investigated. In addition, delta globin gene mutations can reduce the level of HbA2 in carriers of beta thalassemia.

p-563
CLONING AND SEQUENCING OF ABC TRANSPORTER ATP - BINDING PROTEIN ENCODING GENE FROM STREPTOMYCES MINOENSIS

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Bacterial products are secreted or transferred out of the cell via transporter systems such as ABC transporters. Nucleotide analysis and comparison of the Nucleotide Binding Domain (NBD) of the ABC transporter an ATP-binding protein encoding gene from Streptomyces minoensis, by PCR method is described. Following the culture of bacterium the genomic DNA was isolated. The desired DNA fragment was amplified using PCR technique. The amplified DNA was then cloned into pTZ57R/T vector. Competent E.coli: DH5α strain was transformed and the cloned fragment of DNA was then sequenced. The resulting 913 nucleotide chain from ABC transporter ATP-binding protein encoding gene from Streptomyces minoensis was identified and submitted to NCBI under accession number of DQ388679. The blast results of submitted sequence shows high homology in nucleotide and amino acid levels with ABC transporter ATP-binding protein encoding gene/protein from other streptomyces species. Bioinformatics analysis revealed that this amino acid chain contains all of the functional motives of NBD defined in other investigated ABC transporters, ATP-binding proteins.

p-564
STUDY OF BIOLOGICAL ACTIVITIY OF THE HEMAGGLUTININ PROTEIN OF MEASLES VIRUS (AIK-C) USING BACULOVIRUS EXPRESSION SYSTEM

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The baculovirus insect cell expression system has been used as a means of expressing foreign genes. In such a system, the strong and efficient promoter from polyhedrin gene directs transcription of the gene. In our study one of the measles virus membrane proteins, named hemagglutinin (H) which is responsible for receptor binding and hemagglutination activity was expressed by baculovirus expression system, using specific plasmid (pDONR221). Virus particles were extracted by inoculating monolayer Vero cells with the vaccine strain of Measles Virus (AIK-C) followed by 3 freeze-thaw cycles after observing the cytopathic effect (CPE). RNA was extracted from purified viruses and RT-PCR and PCR procedures were performed by specific primers containing recombination sites for cloning of the H gene. The PCR product was then cloned into pDONR221 by BP recombination reaction. Following analysis by restriction enzymes and sequencing, the resulting vector harboring H gene of the Measles Virus was used for expression of recombinant protein in Sf9 insect cells. Synthesis of the H protein in the insect cells infected by recombinant baculovirus was verified by immunoblotting and indirect immunofluorescence (IF), using polyclonal antisera against measles virus raised in goat.The biological activity of recombinant protein was confirmed by hemagglutination test.

p-565
DESIGNING OF EXPRESSION SYSTEMS AND OPTIMIZATION FOR HIGH-YIELD EXTRACELLULAR PRODUCTION OF RECOMBINANT L-ASPARAGINASE II FROM ESCHERICHIA COLI IN SHAKING-FLASK AND BIOREACTOR

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L-Asparaginase II from *Escherichia coli* is an important therapeutic enzyme used in the treatment of leukemia.We have investigated for designing and optimization of an efficient expression system for extracellular production of this enzyme using genetic engineering techniques. The gene coding for L-asparaginase II (ansB), with its native signal sequence, had been cloned in plasmid pHM100 within NdeI and Hind III restriction sites, used as a template for PCR amplifications. The native signal sequence was changed to PelB leader sequence using specific primers that contained NcoI and Hind III sites to facilitate cloning of the gene after the PelB sequence in pET-26b expression vector. 6x His-tag was fused to the C-terminal of enzyme using another reverse primer. Finally 6 different expression plasmids containing ansB gene were constructed and transformed in the expression host E.coli strain BL21 (DE3). Recombinant clones were expressed and assayed for asparaginase activity in periplasm and medium fractions. In order to obtain the highest amount of expression and specific activity, the best recombinant clone with high yield of extracellular expression, was selected by testing its expression in six different culture media. Up to 92 U/ml of recombinant asparaginase was obtained from the extracellular medium of cells induced with 0.1 mM IPTG at late log phase of growth in shaking flask. Up to 152 U/ml of enzyme with high specific activity of 126.6 U/mg was reached at O.D 600 nm for 2 liters Bach-culture of bacteria in bioreactor. Therefore, we developed an efficient extracellular production of Asparaginase II with high specific activity that forms 64% of the purified native enzyme.

p-566
CLONING OF A P5CS cDNA FROM ALEUROPUS LAGOPOIDES

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Salt stress is a major abiotic stress in agricultural plants that affects plant physiology. Halophyte plants complete their life cycle in salty condition. Thus they are useful models to study salt tolerance. *Aleuropus lagopoides*, an Iranian deserts native halophyte, from poacea family is a close relative of bread wheat. One of the most studied salt- tolerance mechanisms in plants is the accumulation of osmoprotectants like proline. The proline level was increased in salt- treated *Aleuropus lagopoides*, significantly. We isolated a 1530 bp partial cDNA using P5CS specific primers of *Triticum aestivum*. The cDNA had 87%, 85%, 84% homology to Zea mays (corn), Triticum aestivum (wheat), oriza sativa (rice) P5CS, respectively. The *Aleuropus lagopoides* full- length P5CS was isolated and cloned using RACE method.

p-567
APPLICATION OF PCR TECHNIQUE FOR IDENTIFICATION OF PENICILLIN G ACYLASE PRODUCING E.COLI STRAINS

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Penicillin G acylase (PGA) is an important enzyme in the bulk pharmaceutical industry. It is used to hydrolyze benzyl penicillin to generate phenyl acetic acid and 6-aminopenicillanic acid. 6-aminopenicillanic acid is a substrate for production of many semi- synthetic penicillins. The PGA gene has been isolated from different organism displays distinct biochemical properties which may be important from industrial aspects. In this study E.coli isolates obtained from environmental and clinical specimens were screened for PGA by PCR technique. Samples from water, soil and clinical specimens were transported to the laboratory and subjected for routine microbiological identification. DNA extracted from different E. coli isolates entered in PCR reactions using primers designed based on conserved region of PGA genes. PCR products from positive samples were purified, cloned and subjected for sequencing. PCR screening identified six PGA positive E. coli among 280 isolates that were collected from different specimens. One of the positive isolates designated number 13, were cloned in pGEM T easy vector and sequenced. The gene encoding a penicillin G acylase from an E. coli isolate had an open reading frame of 2540 nucleotide encoding 846 amino acids. Analysis of sequencing results shows that the PGA gene is highly conserved among E. coli strains.

p-568
GENOMIC CHARACTERIZATION OF ANSB LOCUS IN KLEBSIELLA AEROGENES GENOME BY PCR REACTION

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L-asparaginaseII is an periplasmic enzyme that hydrolyze L-asparagine to asparatate & Ammonia .L-asparaginase have been used in clinical treatment of cancers such as acute childhood lymphoblastic leukemia. Although most bacteria contain L-asparaginase but type II L-asparaginase exists only in some of them.There is no L-asparaginase sequence from klebsiella aerogenes. In this research we chose these bacteria to verify the existance of L-asparaginase type II like gene.This enzyme has been reported that is produced by ansB locus of genome in bacteria. We studied this locus as well as the 500 bp of upstream and downstream sequence by DNAMAN software. We also comparatively studied genome from Ecoli, Shigella, salmonella, Erwinia, and by DNAMAN software. Ten loci with relative similarity were selected and degenerate primers designed for these sites. Then genomic DNA of these bacteria has been extracted with phenol-chloroform method. Our target regions were PCR amplified using designed primers. Then we used gel electrophoresis to observe the expected bands. An internal locus about 600 bp was amplified and its identity was confirmed by PCR reaction using internal nested and external locus amplification.

O-569
MOLECULAR CLONING, CHARACTERIZATION AND EXPRESSION ANALYSIS OF A SALT-STRESS INDUCIBLE GENE (ASR6) FROM AELUROPUS LAGOPOIDES

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Environmental conditions such as salinity causes undesired effects restricting plant growth and productivity. However, there is little knowledge about salt responsive genes in plant. It is useful to clone salt inducible genes to study the mechanisms of plant adaptation to abiotic stresses. *Aeluropus Lagopoides* from poacea family is a halophyte native relative of wheat that grows in salt marsh lands. To understand molecular responses and genes of potential importance to salinity in Aeluropus Lagopoides, transcriptional profiles were been analyzed under saline conditions using cDNA-AFLP method. Many genes (also asr6) are expressed differentially at 10 days after 600 mM NaCl treatment. Asr6 was upregulated and induced during salt treatment. The search of data base indicated that it encodes a transcription factor that functions as a RNA binding protein involved in gene regulation and transcription control during salt stress. Entire length of asr6 has been obtain using

methods such as Inverse PCR and 3' and 5' Rapid Amplification of cDNA Ends (3', 5' RACE) PCR. Semi quantitative PCR by Multiplex PCR method has been carried out to analyze the gene expression pattern under different environmental stresses including dehydration, salinity, hyperosmotic pressure, ABA and salcylic acid. Results suggest that in contrast to a house keeping gene, asr6 plays distinct physiological roles in *Aeluropus Lagopoides* cells under different stress conditions.

p-570
CHARACTERIZATION OF ANSB LOCUS IN CITROBACTER FREUNDII GENOME BY PCR REACTION

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L-asparaginaseII is an periplasmic enzyme that hydrolyze L-asparagine to asparatate & Ammonia. L-asparaginaseII has been used in clinical treatment of cancers such as acute childhood lymphoblastic leukemia. Although most of bacteria contains L-asparaginase but type II L-asparaginase, exists only in some of them.There are no L-asparaginase sequence reported from *Citrobacter freundii*. In this research this bacterium was chosen to survey the existance of a gene similar to L-asparaginase type II.This enzyme is produced by ansB locus of genome in reported bacteria. This locus was studied in addition to 500 bp of upstream and downstream sequence by DNAMAN software. The comparative genomics was investigated by studying genome from Ecoli, Shigella, salmonella, Erwinia, by DNAMAN software. Ten loci with relative similarity were selected and degenerate primers designed for these sites. Then genomic DNA of these bacteria has been extracted with phenol-chloroform method. The target regions were PCR amplified using designed primers. Then gel electrophoresis was performed to observe the expected bands. An internal locus about 980 bp was amplified which its identity was confirmed with internal nested and an external locus amplification by PCR reaction.

p-571
CD40 EXPRESSION IN WEHI-164 CELL LINE

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CD40 is an important mitogenic receptor on B cell. CD40 mRNA was detected in human lung, gingival and synovial dermal by RT-PCR technique. More over the CD40 protein was detected on cultured human fibroblast using anti-CD40 MAb and flowcytometry and on fibroblast dermal tissue section via in situ staining. While the function of CD40 fibroblast is not yet known it seems the CD40 expression is related to cell growth. It might facililate fibroblast proliferation, tissue repair and so on. The purpose of this study was to determine whether mouse fibrosarcoma express CD40, a 50 kD member of TNFR superfamily. Surprisingly we found CD40 expression in Wehi-164 fibrosarcoma by Real time – PCR. But in flowcytometric assay no signal which could be attributed to CD40 expression on cell surface has been detected. It may be a unique phenomenon for fibrosarcoma. Further evalution for mechanism of this event and its role in physiological activity is recommended.

p-572
INTRODUCING A NEW DISULPHIDE BOND IN INTERLEUKIN 2 BY SITE-DIRECTED MUTAGENESIS IN ORDER TO STUDY THE EFFECT OF THIS CHANGE ON BIOLOGICAL ACTIVITY

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Interleukin-2 (IL-2) is a 133 amino acid alpha-helical protein secreted by activated T-cells. This protein has 3 cysteine residues; cysteines 58 and 105 form an intramolecular disulfide bridge, whereas cysteine 125 has a free sulfhydryl group. For structure-function analysis, a cDNA clone encoding biologically active human IL-2 was mutagenized by site-directed mutagenesis to change certain amino acids in the mature protein. In this study mutant proteins with substitution of alanine for cysteine at position 125 and cysteine for leucine at position 18 were produced. In the first mutant we have diminished the free sulphydryl group. This change could probably prevent the formation of mispaired disulphide bonds during the refolding process. The second mutation is designed to introduce a new site for formation of an extra disulphide bond. These analogs were then expressed in E.coli in order to investigate the effect of these changes on biological activity of interleukin-2. The expressed proteins were isolated as inclusion bodies so they had to be refolded and purified before activity assays. Single step purification and refolding was done on Ni-NTA columns and circular dichroism was used for verification of the conformational integrity. The biologic characteristics of each mutant will be tested in the standard murine CTLL-2 assay and the results will be compared to native form.

p-573
ISOLATION OF CEPHALOSPORIN ACYLASE POSITIVE PSEUDOMONAS SP FROM CLINICAL AND ENVIRONMENTAL SPECIMENS

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The cephalosporins belong to the family of β-lactam antibiotics. Cephalosporin acylases are a group of enzymes that hydrolyze cephalosporin C (CPC) and/or glutaryl 7-amino cephalosporanic acid (GL-7ACA) to produce 7-amino cephalosporanic acid (7-ACA). 7 -Amino cephalosporanic acid (7-ACA) is the starting material for the industrial production of most cephalosporin antibiotics (semisynthetic cephalosporins), therefore, cephalosporin acylase is a very

important enzyme for producing semisynthetic cephalosporins. The cephalosporin acylase can be found in several seudomonas sp (such as p. putida, P. cepacia BY21 p. nitroreducens, p.syringae, p. SE83, p.V22, p. SY-77, p. sp.130 ,…) and other bacteria. Therefore, screening of cephalosporin asylase positive pseudomonas is very important. We tested many clinical (patient samples) and environmental (water, soil and hospital environment) samples (about 300 samples) for pseudomonas sp. The samples were cultured in general and selective media, and the routine biochemical laboratory tests (such as oxidase test, oxidation – fermentation test, etc.) were used for diagnosis of pseudomonas sp. In this research, we isolated 160 pseudomonas sp (90 clinical samples, 48 hospital environment samples, 15 water samples, 7 soil samples). All of the isolated pseudomonas species were tested for cephalosporin acylase by PCR method .We designed the suitable forward and reverse primers for PCR, and then we used PCR and gel electrophoresis for screening and selection of cephalosporin acylase positive pseudomonas by analysis of gel electrophoresis patterns. We found 5 cephalosporin acylase positive pseudomonas species from clinical samples. These strains were selected for sequencing and cloning in E.coli and assessment of gene expression in others stages of our study.

p-574
OPTIMIZATION OF FACTORS AFFECTING IN VITRO SOMATIC ORGANOGENESIS AND EMBRYOGENESIS OF ANTHURIUM ANDREANUM

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In vitro propagation of Anthurium has special importance for rapid clonally propagation and disease elimination. In this research micro propagation of *Anthurium adreanum* was investigated through somatic organogenesis and somatic embryogenesis methods. Leaf explants (1×1 cm) of Tera cultivar were used. Segments of leaf were sterilized with 3% NaClO for 30 minutes. Explants were cultured on modified MS media with (0, 0.08, 0.16, 0.24 mg/l) 2, 4–D, (0.5, 1, 1.5 mg/l) BA, 30 g/l sucrose and 8 g/l agar for callus induction. Cultures were maintained in dark at 25˚C. The experiment was conducted as a completely randomized design (CRD) with factorial arrangement and 6 replications. Callus were cultured on MS media supplemented with (0, 0.2, 0.4, 0.6, 0.8, 1 mg/l) BA for somatic organogenesis (shoot induction) in light condition. Best result for callus induction was obtained on the medium containing 0.08 mg/l 2, 4-D and 1 mg/l BA. The media without hormones induced the highest number of shoots. Plantlets were transferred to pots and grown in the green-house. To study somatic embryogenesis, modified 1/2 MS with 30 g/l sucrose, (1, 2,3 , 4 mg/l) 2.4-D and (0.33, 0.66, 1 mg/l) kinetin. Embryo Induction was carried out dark condition. The highest percentage of somatic embryogenesis was observed on a medium containing 3 mg/l 2, 4-D and 0.33 mg/l kin. Conversion and maturation of embryos occurred on the same basal media with 0.4 mg/l BA and 0.25% gel rite at 16 h light. Embryos after germination were transferred to in vivo condition and were sowed on a perlite bed.

p-575
ANALYZING WHEAT SUCCINATE DEHYDROGENASE SUBUNIT II EXPRESSION PATTERN UNDER SALT STRESS

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About one-third of Iranian's area has being affected by salinity. This area is suitable place for adaptation plants to salt stress. In this research we studied expression pattern of a salt stress inducible gene, Succinate dehydrogenase subunit II (SDH II), which previously isolated from Mahooti, One of the Iranian salt tolerant bread wheat using DD-PCR. SDH II encodes an iron-sulfur subunit of SDH. Succinate dehydrogenase is one of the most important enzymes in krebse cycle and electron transport chain. We analyzed its expression pattern in shoots of Mahooti in addition a tolerant and a sensitive one such as Karchia and Chines spring, respectively. Although the SDH II expression level was increased gradually by time in both Karchia and Mahooti, but it was higher in Mahooti even in salt untreated plants. It seems that overexpression the SDH transcripts in salt treated plants have a critical role in sodium detoxifying system.

p-576
EVALUATION OF STABILITY OF THE RECOMBINANT PET21B+PA PLASMID, ENCODING PROTECTIVE ANTIGEN OF BACILLUS ANTHRACIS IN ESCHERICHIA COLI

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The recombinant plasmid pET21+PA that has been deposited at Genbank with accession number EF550209 was constructed by inserting the 1700-bp PA (protective antigen of Bacillus anthracis) recombinant gene into XhoI/HindIII sites of the pET21+ vector under the control of the T7 promoter for highly expressing PA. pET21+PA was transformed into Escherichia coli BL21 strain. The high activity of T7 RNA polymerase could make a powerful expression system for high-level expression of the recombinant proteins. However, during the large-scale production of recombinant proteins, the productivity of a fermentation process is directly affected by many factors, such as plasmid stability, protein production, and culture conditions. In this study, we studied the effects of various culture conditions on the plasmid stability and target protein yield including antibiotic concentrations, the time of induction by IPTG, and the number of successive cultures. The results indicate that the plasmid pET21+PA is stable after the 50th generation completely. The loss of plasmid and structural changes were not detected but the yield of protein production decreased about 10% in 50th generation. These data would be useful for the industrial production of the recombinant PA vaccine and other recombinant proteins.

p-577
PURIFICATION, CHARACTERIZATION AND OVEREXPRESSION OF HUMAN RECOMBINANT ALPHA-1-ANTITRYPSIN IN SACCHAROMYCES CEREVISIAE

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Alpha-1-antitrypsin (AAT) is an important member of the serine protease inhibitor (serpin) superfamily. This glycoprotein consists of carbohydrates (12%) and amino acids (394 units), and has a molecular weight of 54 KD. AAT is secreted into the plasma after being synthesized in the liver. The main function of AAT is the inhibition of the neutrophil elastase and therefore protection of tissues, particularly lung tissue, from the destructive activity of elastase during inflammation. Hereditary deficiency of AAT is associated with the lung and liver diseases, particularly with emphysema as the most remarkable one. Due to its widespread application in medicine recombinant AAT production in various animals, plants and fungal sources has long been an aim of large pharmaceutical companies, Since AAT is glycosylated and has a high molecular weight (MW), any procedure increasing the expression and secretion of the recombinant protein in yeast can be useful in the production of it. Objectives: In this study, AAT was isolated and purified and its characteristics such as molecular weight and inhibitory capacity were calculated after being expressed and overexpressed. Materials and Methods: The yeast *Saccharomyces cerevisiae* strain 2802 and also *Escherichia coli* strain DH5α were used for transformation and propagation of plasmid. The plasmid PYInu-AT was used for transformation of human AAT cDNA into the yeast. Luria-Bertani (LB), Yeast Nitrogen Base without uracil (YNB) and yeast complete medium (YPD) were used for bacterial culture, yeast culture and yeast expression, respectively. Chemical transformation was applied to the plasmid that carried AAT cDNA into the bacteria. Transformation of the recombinant protein into the yeast was performed through a lithium acetate method. SDS-PAGE electrophoresis was carried out to determine AAT molecular weight. Trypsin Inhibitor Capacity was the enzymatic method of choice to assess functionality of the protein. Results: AAT was produced as a secretory protein in S. cerevisiae and overexpresed in fermantor. Introducing the yeast expression media to the fermantor, an overexpression of 9.5 folds was observed for AAT. The molecular mass of the yeast-produced AAT estimated by SDS-PAGE was similar to that of the natural human plasma form. This method can be applied for the commercial production of the synthetic form of human AAT with its native and active characteristic.

p-578
A COMPARATIVE STUDY ON THE EXPRESSION, ACTIVATION AND IMMUNOAFFINITY OF THE NATIVE AND MUTANT STREPTOKINASE

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Streptokinase (SK) is a bacterial protein and commonly used fibrinolytic agent whose efficacy with regard to mortality reduction in patients with acute myocardial infarction (AMI) has been demonstrated in large trials. Since streptococcal infections are common, antibodies (Abs) to SK can be detected in most of adults which are immunized with SK. These antibodies can bind to SK and inhibit its function, when there is need in a further SK therapy. Deletion of the different regions of streptokinase gene can reduce its immunoaffinity. One of these regions is associated to C-terminal region of streptokinase gene which its deletion has no effect on the activity of this protein. In this study the mature streptokinase gen was isolated from *Streptococcus equisimilis* H46A ATCC12449 and cloned into pGEX-4T-2 vector. The mutant streptokinase gene amplified by deletion PCR method, cloned into the same vector and transformed into E.coli BL21 (DE3) host cell. Protein expression was induced by adding IPTG. The expression was checked by SDS-PAGE analysis. The activity of mature and mutant SK was measured by plasmin hydrolysis of chromogenic peptidyl anilide substrate (S-2251) and monitored at 405 nm. Although mutant streptokinase lacks the C-terminal 126 nucleotides, but no significant changes in its activity was observed in comparison to the expressed native SK. A direct binding assay was performed in order to compare the mutant SK with mature SK protein regarding to their capacity for binding to human anti-SK Abs present in sera from patients after streptokinase therapy. Results showed that as it was expected, human anti-SK Abs binds to mutant SK less than native SK.

p-579
SEQUENCING OF ITS-1 REGION OF TYLODELPHYS CLAVATA (VON NORDMAN 1832)

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Parasites morphologically consistent with *Tylodelphys clavata* were collected from the vitreous humor of *Chondrostoma regium*, a native fish species that inhabits in Choghakhor lagoon in Chaharmahal va Bakhtyari province. Samples examined by PCR of the ITS-1 region using 1 pair F45 and R4 Primers. The ITS region is now the most widely sequenced DNA region in most of the organisms. It has typically been used for molecular systematics at the inter species and even within species levels. The expected amplification band (560-bp) was observed on agarose gel. The amplified sample was cloned and then sequenced to assess accuracy of result and to confirm obtained data. The blast result confirmed that amplified product is corresponding to *Tylodelphys clavata* without any amplified artifact or carry over contamination. It should be noted that the homologous sequences were found by blast search were not the same those previously had deposited in GenBank by Anandan et al. that might indicates the heterogeneity of studied gene among different variants of this parasite in Iran.

p-580
STUDY ON GENETIC DIVERSITY OF FARS NATIVE CHICKENS USING RAPD MARKERS

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Genetic diversity of Fars native chickens population was studied using RAPD primers. The DNA samples extracted by modified salting-out method. The genomic DNA amplified by using 10 RAPD randomly primers by polymerase chain reaction (PCR). From 130 obtained bands 63 were polymorphic. Therefore there is 48.5% polymorphism in this population. The most and least polymorphic bands obtained by OPN-16 primer with 13 bands and OPX-20 primer with 1 band, respectively. Length of amplified fragments by all primers was between 237-3240 base pairs. The smallest and largest fragments produced by OPT-17 primer and OPN-16 primer were 237 bp, and 3240 bp, respectively .The genetic diversity index of population was 0.160. The low genetic diversity of population has been proposed that increase the effective breeding number (Ne), the rate of effective breeding (Ne) to the number of population (N). Association control prevented diversity reduction and therefore inbreeding increased.

Molecular Genetics

O-581
IDENTIFICATION, ISOLATION, CLONING AND SEQUENCING A PARTIAL ANNEXIN GENE FROM AUREOBASIDIUM PULLULANS

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Bacground: Annexin is the common name for genes and proteins that identified as calcium-dependent phospholipid-binding proteins but recently has been recognized more complex set of functions for these superfamily of proteins in vesicle trafficking, cell division, apoptosis, calcium signalling, mineralization, crystal nucleation inside the extracellular organelles-matrix vesicles (MVs) and growth regulation. Material & methods: In the present work Aureobasidium pullulans strain PRAFS8 genomic DNA was extracted and using designed primers from highly conserve region of anexxin genes of Aspergillus fumigatus and Aspergillus niger a 800 bp PCR product obtained from degenerated PCR. The 800 bp PCR product was gel purified and cloned into E. coli Top-10F´ (Stratagene) using the pGEM®-T easy vector system (Promega) and standard cloning procedures. From grown transformed E. coli Top-10F´ cells, pGEM®-T easy vector was extracted and the presence of expected insert into plasmid, was confirmed by digestion of plasmid by Eco R1 restriction enzyme. Gel purified 800 bp band was sequenced and submitted at NCBI gene bank with accession No.: AY848856. A phylogenetic tree for obtained partial gene of annexin was drawn using bioinformatic software in order to understanding the evolutionary relationship of annexin genes between some microrganisim. Also southern analysis of 800 bp PCR product using digoxigenin labeled probe with DIG-High Prime DNA Labeling kit (Roche) demonstrated the probability of two copies of annexin genes existence in the A. pullulans genome. Results: This work fro first time was presented the presence of annexxin gene in yeast like fungi and this result is important due to exist of this superfamily of genes in moulds but not in yeasts. Conclusion: We emphasize for future additional work to clone and sequencing the full length of annexin gene(s) from A. pullulans and also additional studies for this gene expression and annexin mRNA transcription to understand the effective factors for expression of annexin.

p-582
THE CLONING OF BOVINE LEUKEMIA VIRUS TAX GENE IN IRAN

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Bovine leukemia virus (BLV) is a member of the family Retroviridae, genus Deltaretrovirus. That has three important gene including gag, pol and env and number of replicating regulator genes such as Tax, Rex, R III and C IV. For clonizing the Tax gene of this virus, the first step PCR product of Tax gene of BLV strains isolated in region of Iran and BLV-FLK strain were cloned in to a pCR 4-TOPO vector (TOPO T/A Cloning kit, Invitrogen), then insert were digested by Bam HI and Xho I restriction enzymes and cloned in to pET-32 (a) as an expression vector. With considering the noticeable increasing of BLV virus infectious in Iran and the need for controlling the infection or disease via vaccination, expression gene and providing the Tax recombinant protein combination can be of available aims not for away in the future.

p-583
CELL CYCLE ANALYSIS OF THE CD133 CELLS ISOLATED FROM UMBILICAL CORD BLOOD

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During interphase of the cell cycle when the cell is not dividing the DNA in the nucleus is in use. In the S phase DNA is duplicated and nuclear division occupies a very small part of the cycle. Umbilical cord blood cells exhibit high proliferative capacity leading to a large expansion of cell density in appropriate cell culture conditions. The cell cycle status and responsiveness to in vitro cytokine stimulation is critical defining strategies for umbilical cord blood stem cell expansion. The aim of this study was to determine the cycling status of CD133 cells at various culture conditions by flow cytometry analysis. Human umbilical cord blood cells were obtained from pregnant women during their full - term normal

delivery with informed consent. The mononuclear cells fraction was separated by Ficoll Paque density centrifugation. An immunomagnetic device was used for cell separation. Cells were passed through a Mini MACS column retained in a magnetic field, and the column was washed with PBS to remove unbound cells. After separation, CD133 cells were seeded in Iscoves Modified Dulbeccos Medium (IMDM) with different serum concentrations and were stimulated with SCF (100 ng/ml), IL-3 (50 ng/ml) and Il-6 (50 ng/ml). Cell cycle analysis was carried out using Cycle Test PLUS DNA Reagent Kit. Cycling condition of fresh samples in different culture media was investigated after isolation and as well as 1 and 2 weeks after culturing. Fluorescence intensity was measured using a FACS Calibur, four- color scan, equipped with argon laser at 448 nm wavelength and red diode laser at 635 nm, and analyzed using Cell Quest. The study showed that 96.75 ± 0.58 % of CD133 cells were in G0/G1 –phase and 2.02 ± 0.38 % were in S-phase immediately after separation. Also, the source and concentration of the serum used is an important factor on cell populations. Cell cycle status may be an important factor for defining cultivation strategies for stem cell expansion.

p-584

PHYLOGENETIC AND MIGRATION ANALYSIS OF IRANIAN POPULATIONS BASED ON THE CONNEXIN-26 MUTATIONS

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Mutations in the coding region of connexin 26 (Cx26) have been identified as major cause of autosomal recessive non-syndromic hearing loss in the several populations. In this study we have investigated the prevalence of the GJB2 gene mutations by using direct sequencing method. Eight different mutations in 13 individuals out of a group of 51 patients were detected. These mutations were combined with previously reported mutations identified in other populations with different ethnic backgrounds from different parts of Iran. Altogether 33 mutations related to deafness in Cx26 have been detected. These findings were compared with data from other populations. Iranian population had more similarity (33.3%) with European and Pakistani (30.3%) mutations than other countries. S86 were detected in all ethnic groups of Iran and we confirmed the Asian origin of W24X mutation. The analysis of phylogenetic tree showed the V27I, E114G and V27I+E114G mutations were common ancestors as verified by three methods, UPGMA, Neighbor–joining trees and optimal distance base Minimum evolution tree.

p-585

GENE EXPRESSION PROFILES IN MOUSE LIVER CELLS AFTER EXPOSURE TO DIFFERENT TYPES OF RADIATION

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Purpose: The liver is one of the target organs of radiation-induced cancers by internal exposures. In order to elucidate radiation-induced liver cancers including Thorotrast, we introduce a new approach to investigate in vivo effects of internal exposure to alpha particles. Materials and methods: Adopting boron neutron capture, we separately irradiated Kupffer cells and endothelial cells in mouse liver in vivo and the changes in gene transcriptions were analyzed by an oligonucleotide microarray. Results: Differential expression was defined as more than 3-fold for up-regulation and less than 1/3 for under-regulation, compared with non-irradiated controls. Of 6,050 genes examined, 68 showed differentially expressed genes compared with non-irradiated mice. Real-time polymerase chain reaction (PCR) validated the results of the microarray analysis. The pattern of altered gene expression was different between exposure to alpha particles and gamma-rays. Gene expression profiles revealed that the liver was in an inflammatory status characterized by up-regulation of positive acute phase protein genes, irrespective of the target cells exposed to radiation. In comparison with chemical and biological hepatotoxicants, inductions of Metallothionein1 and Hemopexin, and suppressions of cytochrome P450s are characteristic of radiation exposure. Conclusion: Anti-inflammatory treatment could be helpful for the prevention and protection of radiation-induced hepatic injury.

p-586

ASSESSMENT OF GM-CSF RECEPTORS BY REAL – TIME RT - PCR ON VARIOUS CELL LINES EXPRESSING HIGH AND LOW AFFINITY RECEPTORS AND THEIR RELATION TO CYTOTOXIC EFFECT OF CHIMERIC PROTEIN (STXA1-GM-CSF).

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Immunotoxins, comprised of both the cell targeting and the cell killing moieties, are the new approach for targeted therapy of human disease. In all immunotoxins that GM-CSF has been used as cell targeting only cell lines expressing high affinity receptor have been used for cytotoxicity studies. In the present study, various cell lines expressing high and low affinity receptors were used for the assessment of cytotoxic effect of hybrid chimeric protein. The expression of GM-CSF receptor (GM-CSFR) was quantified by real-time RT- PCR. K562 and THP1 cell lines expressing high affinity receptor and MC-7, PC-3 and DU145 expressing low affinity receptor were used in this study. The chimeric hybrid protein was found to be toxic for various cell lines used and cytotoxicity was more effective in cell lines bearing high affinity receptor. Overall our result showed that the recombinant hybrid protein could have wide range of application on various cancer cell lines even cells bearing low affinity receptor for GM-CSF.

p-587
FREQUENCY OF 844INS68, CYSTATHIONINE ß - SYNTHASE POLYMORPHISM AS AN ANTHEROPOLOGICAL MARKER TO DIVERSITY OF SOUTHERN IRAN POPULATION FROM OTHER POPULATIONS

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Iranian population with an Indo-European origin is one of the oldest populations in the world. Historical evidence suggests the close similarity in the origin of Iranian, European and north Indian population. However, there are few anthropological and genetic evidences on this subject. This study, which is the first report from Iran, was performed to investigate the genetic origin of Iranian population using a polymorphism in Cystathionine beta synthase (CBS) gene known as 844INS68bp. Genomic DNA was extracted from the whole blood of 480 healthy normal blood donors referred to Fars Blood Transfusion Center, using a salting out method. The fragment containing 844INS68bp was amplified, the normal fragment was 174bp and the fragment containing the insertion was 242bp in length. Results indicated that 418 (87.08%) out of 480 individuals had a normal (N/N) genotype, 59 (12.29%) individuals were heterozygote (N/I) and 3 (0.63%) had homozygote a mutated genotype (I/I). The total frequency of 844INS68bp allele was found 6.8%, which is similar to with the reported in White Caucasians. Comparison of the genotype of this study with the polymorphism in other populations revealed that Southern Iranian population has a great similarity with other Caucasians populations, especially South Italy and North America while differed from East Asian and African populations. These results are in agreement with the result of other studied polymorphisms. Therefore, despite the great admixture of Iranian population with the neighboring non-Caucasian populations by time, Iranian population still share a genetic background with other Caucasian populations.

p-588
DIRECT HAPLOTYPING OF BI-ALLELIC SNPS USING ARMS AND RFLP ANALYSIS TECHNIQUES

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Determination of single nucleotide polymorphisms (SNPs)-based haplotypes is an important and rapidly growing approach in association studies and gene mapping. It is more informative tool than genotyping because of converting bi-allelic forms into multi-allelic patterns. The aim of this study was to improve a combinatory method for direct haplotyping of three SNPs, using amplification refractory mutation system (ARMS) and restriction fragment length polymorphism (RFLP) analysis techniques. The ARMS technique was applied to separate two alleles. Then, Nested-PCR reaction and RFLP analysis were performed for determination of the haplotypes of polymorphic positions. The method requires prior information of polymorphic sites. Compared with procedures based on allele-specific PCR methods this method has the advantage of using less allele-specific primers. We determined haplotypes of three SNPs in promoter of the *PON1* gene and found the method is suitable for evaluation of SNPs variations on each allele. Moreover, the procedure is feasible and has a high specificity for large samples in population studies.

p-589
PREMATURE CENTROMERE SEPARATION OF CHROMOSOMES IN A WOMAN BEING EXPOSED TO HIGH-LEVEL NATURAL RADIATION IN RAMSAR

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In a cytogenetic study on the effect of high background natural radiation in Ramsar, north of Iran, a woman had an acentric like C group chromosome showing the premature centromere separation frequently in the peripheral lymphocytes. By using Fluorescent In Situ Hybridization (FISH) method, this unusual chromosome was verified as being as an X chromosome. Analysis of the 2038 cells revealed that these unusual X chromosomes had ability to replicate. This replication was associated with non-disjunction leading to aneuploid cells. Induction of the premature centromere separation (PCS), which is seen in the aged women, is discussed in relation to the effect of the chronic exposure to low dose.

p-590
MANNOSE - BINDING LECTIN GENE AND PROMOTER POLYMORPHISM IN RENAL TRANSPLANT RECIPIENTS

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The aim of present study was to determine the distribution of the alleles of Mannose-binding Lectin (MBL) gene codon 52, 54 and 57 and promoter variants H/L, X/Y, P and Q in renal transplant recipients in comparison with normal subjects. Another objective of this study was to seek the correlation between these variants and diseases that cause renal dysfunctions. One hundred white blood samples prepared from thirteen renal recipients were compared with those of one hundred and twenty normal subjects from Azarbaijan population of Iran. MBL genotypes were investigated by polymerase chain reaction and restriction fragment length polymorphism. Allelic and genotypic frequency of the polymorphism at position- 550, -221,+4 and at codon 52 , 54 and 57 did not show statistical differences between recipients and controls but significant frequency of allele B (codon 54) and Lx haplotype of promoter was observed in patients with Lupus Erythematosus ($p<0.05$) and infection sources of renal dysfunctions ($p=0.003$). In conclution, Our findings provide evidence that presence of different alleles and haplotypes that cause low concentration of MBL in serum is a risk factor for the severity of systemic Lupus Erythematosus and susceptibility to renal infections that cause renal dysfunction.

O-591
A NEW METHOD FOR SCREENING OLIGONUCLEOTIDES ON SOLID SUPPORT

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A new solid support for oligonucleotide synthesis was developed. Coupling of the oligonucleotides is done using standard synthesis methods. The solid support was used for synthesizing oligonucleotide libraries, directly bound via a phosphoamidate linkage. This is especially suitable for biomolecular screening and for all applications, requiring the oligonucleotides to remain bound to the solid support after synthesis. The research presented herein is dealing with the construction of an oligonucleotide library and the interaction of it with human factor Xa from the blood coagulation cascade. Selection of the particles was carried out using a micromanipulation system with fluorescence detection. Sequencing of the oligonucleotide by MALDI-TOF MS after cleavage from the solid support was made from one particle and an oligonucleotide was found, binding strongly to human factor Xa. The properties of this solid support, its possibilities and limits were investigated and its applications in modern diagnostics were figured out.

p-592
USE OF THE KERATIN-14 GENE PROMOTER FOR EXPRESSION OF HUMAN COAGULATION FACTOR IX IN CULTURED EPIDERMAL KERATINOCYTES

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Keratinocytes are suitable models for ex vivo gene expression strategies for the systemic release of a protein. To establish a keratinocyte-specific expression system for studying the keratinocyte–mediated expression of a trans-gene product a human factor IX (hFIX) expressing plasmid was constructed by inserting the hFIX-cDNA downstream to a 2200bp keratin 14 (k14) gene promoter in a pcDNA3-based plasmid. Human epidermal keratinocytes isolated from neonatal foreskin were cultivated in keratinocyte serum-free media and transfected with the recombinant plasmid. The K14-promoter-driven expression of recombinant hFIX was evaluated by performing coagulation test as well as enzyme-linked immunosorbent assay on the cultured media collected from the transfected cells. The rhFIX expression was also confirmed by performing RT-PCR, dot and Western blotting. The results support the potential of keratinocytes for the expression of biologically active rhFIX. The highest value of FIX Ag measured by ELISA (as displayed by 106 cells after 24 hours) was estimated to be 180 ng/ml, which is still comparable to those obtained by other groups who used regulatory elements from CMV (250 ng/ml), retrovirus (830 ng/ml) or moloney murine leukemia virus (600 ng/ml) for the expression of hFIX . The current keratinocyte-based expression system has provided means for further bioengineering strategies to improve the expression efficiency of hFIX in keratinocytes.

p-593
CHROMOSOMAL CHEMOSENSITIVITY OF F3B6 HYBRIDOMA CELL LINES IN COMPARISON WITH ITS PARENTAL NORMAL CELL LINES

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Somatic cell hybrids are important cells that provide a very useful tool to produce monoclonal antibody and to map a number of genes and functions.These cells are obtained from the fusion of normal lymphocytes with myeloma cell lines. When applied to cells from different species, interspecific somatic cell hybrids are formed. Following the fusion, two genomes locate in different condition (new hyaloplasm).This study was carried out to investigate whether this new condition affects chromosome sensitivity to mutagens by

analysing chromatid and chromosome aberrations in respons to two chemotherapic drugs , bleomycin sulfate and actinomycin D. A hybrid cell line, F3B6, and its parental cell lines, as normal cell lines, were treated with these drugs in G1 or G2 phases of the cell cycle and mitotic cells were analyzed accordingly.Results show that chromatid and chromosome aberrations induced by both these drugs in hybridoma cell line are more than those in its parental cell lines. These results indicate that chromosomes in hybridoma cells are more sensitive to mutagenic and clastogenic effects of chemotherapeutic compound than normal cells.

p-594
NUCLEOTIDE SEQUENCE OF CODING REGIONS OF AN IRANIAN BYDV-PAV ISOLATE

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Barley yellow dwarf viruses (BYDVs-PAV) cause serious losses to cereal crops worldwide. These viruses are phloem limited, belong to Luteoviridae and are transmitted by aphids in a circulative manner. Previous results revealed the wide distribution of these viruses throughout Iran. Among them, BYDV-PAV is the most prevalent virus in Iran. However there is limitted information in regard to its genetic variability. In this study complete nucleotide sequence of genes of an Iranian BYDV-PAV was determined and compared with the sequences of other BYDV-PAV isolates which are present in the GenBank. An ELISA-positive barley plant was used as the source of virus for total RNA extraction and further analysis. RT-PCR was carried out using eight BYDV-PAV specific primers. The amplified fragments were inserted into pTZ57R/T and cloned in Escherichia coli (DH5α). Amplified segments were assembled to obtain near-full length of the genome, comprising 5180 nucleotides. According to phylogenetic analysis of the amplified fragment, the Iranian isolate (ESP-1149 from Kerman province) grouped with other BYDV-PAV isolates and showed 93-94% similarity with BYDP-PAV, EF521834 and BYDVPAV-JAP from GenBank. Its similarity with PAS PAV-129 was only 83%.

p-595
ASSESSING CELL DEATH: TRYPAN BLUE STAINING OR DNA FRAGMENTATION ANALYSIS. WHICH ONE IS MORE SENSITIVE?

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Apoptosis or programmed cell death is an important cell cycle mechanism which is used to eliminate harmful cells. To estimate cell death by DNA fragmentation study there are many kinds of laboratory tests in which some like ISNT (In Situ Nick Translation) TUNEL(- TdT – mediated X-dUTP nick end labelling) use labeled dNTP and some are hallmark of apoptosis like DNA fragmentation analysis by gel electrophoresis. In this study to assess cell death in 13 different cell lines undergoing normal death, we were employing two technique of Trypan blue staining and DNA fragmentation study. Cell-lines were ordinary cultured, and then their numbers was adjusted and incubated without media exchange till cell death happened. Our results showed thet Trypan blue staining is still a more reliable technique to estimate cell death particularly in early stages of death and DNA fragmentation may be not observed even in last stages of cell death.

p-596
STUDY OF THE PRODUCTION OF IgA AFTER THE USE OF ORAL BCG ENCAPSULATED IN ALGINATE MICROSPHERES FOR DNA VACCINE PRODUCTION

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Study of production IgA after use oral BCG encapsulated in alginate microsphere to make DNA Vaccine.DNA Vaccinations are new methods which primarily stimulate the systemic immune responses. Also the useof adjvatants are more useful with highly specific immunizations. One of the best adjuvants is BCG and is easily used when administered orally with a segmrnt of the immune gene of viruses and bacteria. First we have to identify the proper BCG for oral use. In the present study, BALB/c mice were vaccinated orally with BCG encapsulated in alginate microspheres, then IgG and IgA levels in sera and lung homogenates, and DTH response were compared with those of mice vaccinated with free BCG by subcutaneouse route. Mice immunized with encapsulated BCG and those immunized subcutaneously with BCG developed comparable DTH responses. IgA level in lung homogenate was significantly higher in the group immunized with encapsulated BCG than the group immunized with BCG subcutaneously. IgG level in the lung homogenate was equal in the two vaccinated groups. Serum IgG level was significantly higher in the subcutaneously immunized group than the group orally immunized with BCG, however immunization with BCG either orally or subcutaneously prouduced the same amount of IgA in serum. Our data indicate that oral administration of BCG in alginate microspheres results in both systemic and mucosal immune responses, and oral BCG could be employed to make DNA vaccine.

p-597
DEVELOPMENT OF AN ELECTROCHEMICAL BIOSENSOR FOR DETECTION OF THE PCR APPLICATION OF HUMAN INTERLEUKIN-2 ENCODING DNA

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Interleukin-2 is an important cytokine secreted by helper T lymphocytes with some clinical applications including melanoma) and renal cell carcinoma. DNA biosensors based on nucleic acid hybridization processes are rapidly being developed towards the goal of rapid and inexpensive diagnosis of genetic and infectious diseases. Due to their high

sensitivity, small dimensions, low cost, and compatibility with microfabrication technology, electrochemical transducers are often being used for detecting DNA hybridization event. In this study, an electrochemical biosensor for the voltammetric detection of PCR product of human interleukin-2 encoding DNA is described for the first time. For this purpose, a DNA biosensor was developed that relies on the immobilization of a 20-mer single stranded oligonucleotide related to the human interleukine-2 gene (IL-2p) as the probe on the pencil graphite electrode (PGE). The guanine oxidation signal was monitored using anodic differential pulse voltammetry (ADPV). Then the electrochemical detection of hybridization events between complementary DNA (cIL-2) as the target DNA and IL-2p was successfully achieved. The selectivity of the biosensor was studied using noncomplementary oligonucleotides. The ability of the electrode in discriminating complementary oligonucleotides from non-complementary oligonucleotides was also approved. Having confirmed the ability of the biosensor in detection and discrimination of the complementary oligonucleotides, IL-2 PCR product detection was tested. The results showed that PCR amplified IL-2 DNA can be detected on the basis of electrochemical signals. Numerous factors affecting the target hybridization were optimized to maximize the sensitivity of the detection. Our results clearly showed that IL-2 PCR product can be selectively detected using PGE DNA biosensors.

p-598
INVESTIGATION OF DNA INTERACTION WITH PALLADIUM CHLORIDE - N, N-DIMETHYLTRIMETHYLENE DIAMINE (Pd Cl_2- LL) BY FLUORESCENCE AND UV ABSORPTION SPECTROSCOPY

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Over the past decades, there has been substantial interest in the deoxyribonucleic acid (DNA) binding properties toward a number of metal complexes, with the aim to develop novel reagents, which can control genetic information and/or prevent the growth and replication of cancerous cells through the inhibition of transcription. The necessary requisites that such complexes should obviously possess, are to be stable, inert in biological environment, and water-soluble. Despite a considerable amount of the literature on metal complex interactions with DNA, the knowledge of the nature of binding of these complexes to DNA and their binding geometries has remained a subject of debate. In this work, the interaction of native calf thymus DNA with the Pd(II) complex, PdCl2(LL) (LL = chelating diamine ligand: N,N-dimethyltrimethylene diamine), in 10 mM HEPES(4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) aqueous solutions at neutral pH, has been monitored as a function of metal complex-DNA molar ratio by UV absorption spectrophotometry, and fluorescence spectroscopy. The results support two modes of interaction. In particular, this complex showed absorption hypochromism and then hyperchromism. The binding constant has been determined using absorption measurements and found to be 2.69×10^{3} M^{-1}. This low value is indicative of outside bonding. As evidenced by the increasing of the fluorescence of methylene blue-DNA solutions in the presence of increasing amounts of metal complex, PdCl2(LL) is able to displace the methylene blue intercalated into DNA, but not completely which indicates partial intercalation.

p-599
DETECTION OF MUTATIONS IN A GENE RESPONSIBLE FOR CUTANEOUS LEISHMANIA CHEMOTHERAPY

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Leishmania species are distributed world wide. During its life cycle, the parasite goes through two developmental stages, the promastigote form of the parasite resides in the intestinal tract of the insect vector while the amastigote form of the parasite resides in macrophages and other mononuclear phagocytes in the mammalian host. 10-15 millions of people have clinical symptoms of leishmaniasis and 400000 new cases are diagnosed each year. The 20 or so infective species and sub species of the parasite cause a range of symptoms, some common (fever , malaise, loss of weight, and anemia) and some specific (swelling of the spleen). The pentavalent antimonial drugs pentastom and glucantim are the first line treatment for leishmaniasis and resistance to these drugs is a serious problem. The systemic analysis of drug resistant mutants should be useful in trying to delineate the drug target. In order to try to understand resistance mechanisms in leishmania we have selected two leishmania species for detection of resistance to sodium stibogluconate. We designed a study for detection of mutation in a gene corresponding to the chemotherapy of leishmaniasis. A primer set was designed for amplification of a segment of leishmania mdr1 gene by PCR. PCR products was scanned by CSGE method and doubtful bands were sequenced.

p-600
DETECTION AND PRESENTATION OF HUMAN PERF15 GENE

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PERF15 is a 15.06 KD protein that specifically expressed in the testis. In 1994 Oko and colleagues introduced this protein as a member of lipid binding protein and named Testis Lipid Binding Protein (TLBP). PERF15 has 61% and 58% amino acid sequence similarity to Myelin P2 and Adipocyte Lipid Binding Protein, respectively. This protein is located in sperm cells between the nucleus and acrosome in the region called perinuclear theca. PERF15 and other protein of perinuclear

theca are involved in attachment of nucleus to acrosome. PERF15 plays an important role in fertilization. It protects the nucleus and equatorial segment from vesiculation during the acrosome reaction. Study in rat and mouse has shown that PERF15 is not only expressed in pachytene spermatocytes and spermatids but is also present in apoptotic germ cells. PERF15 has a role in both apoptosis and spermatogenesis. The study of this protein can clarify the etiology of some cases of infertility. The purpose of this research is to prove the existence of PERF15 in human. First, we amplified human gene using specific primer designed based on rat PERF15 gene. Then using sequence information from NCBI we designed specific primers for human PERF15 cDNA. PCR amplification with human specific primers produced two fragments with a size of 450 bp and 400 bp long. We think one of the PCR bands is human PERF15 -specific PCR product.

p-601
UPREGULATION OF NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN IN β TALASSEMIA PATIENTS

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Beta thalassemias are a heterogeneous group of inherited anemias resulting from reduced or absent synthesis of beta globin chains of hemoglobin A. One of the major consequences of this genetic disorder is iron overload due to ineffective erythropoiesis and premature hemolysis in the plasma and in the major organs. The most anemic patients require regular blood transfusions, which exacerbate their iron overload and result in damage to vital organs. Several studies have reported oxidative status in beta thalassemia patients. It is believed that iron plays critical role in the formation of reactive oxygen species (ROS). We found induction of Lcn2/NGAL expression under oxidative stress condition recently. We hypothesize that NGAL should be upregulated in beta thalassemia patients because of oxidative stress conditions. The assessment of NGAL expression was performed by Real time RT-PCR and ELISA. Our results showed that NGAL was upregulated in beta thalassemia patients compared with normal controls. The upregulation might play an important role in decreasing ROS or iron in beta thalassemia patients.

p-602
STUDY OF MICROSATELLITE MARKERS IN ISFAHANIAN NATIVE CHICKENS BY PCR

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Polymorphisms of Isfahanian native chickens were evaluated using ten microsatellite markers. Nine microsatellite loci were found to be polymorphic, but one of them was monomorph (MCW0216). All microsatellite loci deviate from the Hardy–Weinberg equilibrium. Heterozygosity and the polymorohism information content (PIC) were calculated to determine the genetic variation. Of the nine polymorphic loci, actual and effective number of alleles (na, ne) per locus ranged from 2 to 5 and 2 to 3.9014, respectively. PIC values were between 0.3750 to 0.6972 per locus (except monomorphic locus), and average of PIC based on 10 microsatellite was estimated as 0.4897. The heterozygosity ranged from 0.5 to 0.7437 per locus. Considering monomorph loci the average heterozygosity was 0.5613. In general, it can be concluded that Isfahanian native chickens have approximately low genetic diversity. Considering that the native chickens are endangered for breeding one should design a good program for the best conservation of genetic resources.

p-603
ASSOCIATION OF XMNI POLYMORPHISM IN Gγ-GLOBIN PROMOTER WITH THE LEVEL OF HB F AND G γ CHAIN AND THE Gγ: Aγ RATIO IN β-THALASSEMIA MAJOR AND INTERMEDIA PATIENTS FROM KERMANSHAH

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β-thalassemia is the most common single gene disorder in Iran and more than 25,000 affected individuals have been reported. The pathophysiology of thalassemia is almost entirely due to the imbalance of globin chain synthesis. It has been reported that in patients with β-thalassemia the level of Hb F is increased and better response to hydroxyurea is achieved in the presence of Xmn I polymorphic site. We studied one hundred ninety seven β-thalassemia major and intermedia patients from the Kermanshah province of Iran. The XmnI polymorphic site at the 5´ region to Gγ gene was determined by PCR-RFLP procedure. The Hb F level was determined by electrophoresis method. The levels of Gγ and Aγ chains were detected by high performance liquid chromatography (HPLC). We found that in β-thalassemia patients in the presence of XmnI polymorphic site, either on one or both chromosomes, the level of Gγ chain (71.4% and 74.8%, respectively) and Gγ: Aγ ratio (2.5 and 3, respectively) are significantly increased (P=0.001) in comparison to absent XmnI site (66.7% and 2, respectively). Furthermore, a non-significant increase in Hb F level was observed in the presence of Xmn I polymorphic site on both chromosomes (97.1%) in comparison to the absent site (94.4%). Our results indicate a strong association between presence of Xmn I polymorphic site and the increased expression of Gγ gene in β-thalassemia patients. This finding could be useful to reduce the severity of clinical symptoms and management of β-thalassemia intermedia patients.

p-604
PREVALENCE OF FACTOR V LEIDEN (G1691A) AND PROTHROMBIN (G20210A) MUTATIONS AMONG KURDISH POPULATION FROM WESTERN IRAN

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The mutation in the factor V (FV) G1691A, known as factor V Leiden, and prothrombin (FII) gene G20210A are the two most prevalent causes of inherited thrombophilia. The present study reports the prevalence of factor V Leiden and the prothrombin G20210A gene mutations among healthy population of Kurdish ethnic background in Western Iran. Four hundred thirty-four healthy unrelated individuals, 255 male and 179 female with a mean age of 28.7±15.5 from the Kermanshah Province of Iran were studied for prothrombin G20210A mutation. The factor V Leiden mutation was studied in 404 healthy individuals, of whom 232 were male and 172 were female. The factor V Leiden and prothrombin G20210A were detected by PCR-RFLP method using Mnl I and Hind III restriction enzymes, respectively. Among 434 individuals studied for prothrombin G20210A mutation, seven carried this mutation as heterozygous (4 female and 3 male), giving a prevalence of 1.6% and an allele frequency of 0.8%. No homozygous prothrombin 20210AA was found. Factor V G1691A mutation was detected as heterozygous in 11 out of 404 healthy individuals (5 female and 6 male) and as homozygous in one male indicating a prevalence of 2.97% and allele frequency of 1.6%. Our results indicated that, the factor V Leiden and prothrombin G20210A mutations are not rare among Western population of Iran. Also, studying the relationship between venous thrombophilia and these mutations might suggest a causal effect in Western Iran population.

p-605
THE STUDY OF CORRELATION BETWEEN OSTEOPOROSIS AND ESTROGEN RECEPTOR-ALFA GENE POLYMORPHISM IN IRANIAN WOMEN

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Osteoporosis is a common disease in which bones become prone to fracture as a result of loss of bone mineral density (BMD). The discovery that inactivation of Estrogen Receptor (ER) gene is linked to low BMD, put ER-alfa in the family of genes which trigger osteoporosis. In the present study, we grouped 200 pre- and post-menopausal Iranian women, aged 35-80 yr, according to the genotypes of the ER-alfa gene intron1 PvuII or XbaI polymorphisms, where upper case and lower case letters of Pp (PvuII) and Xx (XbaI) indicates the absence or presence of related restriction sites in RFLP experiments. The frequencies of the ER-alfa genotypes were almost similar to previously published genotype frequencies in European and East Asian populations. However, we detected three genotypes of PpXX, ppXX and PPxx at a very low frequency in the study population. According to our statistical results, we could not observe any significant relationship between BMD and intron1 RFLPs. This research suggests that other factors accompanying ER-alfa gene polymorphisms might cause osteoporosis. A further study needs to be carried out in order to investigate the correlation between BMD and (TA) n repeat polymorphisms upstream of exon 1.

p-606
PREVALENCE OF XMNI POLYMORPHIC SITE AT THE 5´ REGION OF THE Gγ-GLOBIN GENE IN β-THALASSEMIA MAJOR AND INTERMEDIA PATIENTS FROM KERMANSHAH

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Beta thalassemia is the most common inherited blood disorder, affecting synthesis of the beta globin chain of hemoglobin. The type of β-thalassemia mutation affects on the β-globin chain synthesis that present as β°، β + and β++ thalassemia. Studies have shown that, in the presence of XmnI polymorphic site, the response to treatment with hydroxyurea would be better. For this study one hundred ninety seven beta thalassemia major and nine-beta thalassemia intermedia patients were recruited from shahid fahmideh hospital in Kermanshah. DNA was extracted by phenol-chloroform method and the XmnI polymorphism at the 5´ region to Gγ globin gene was determined by PCR-RFLP procedure. Analysis of XmnI polymorphic site revealed that among β-thalassemia major patients there were 32 (16.3%) homozygous for XmnI (+/+) and 44 (22.3%) were heterozygous for XmnI (+/-). In patients with β-thalassemia intermedia 55.6% of patients had two XmnI sites and 22.2% had one XmnI site. Since there is a strong correlation between the presence of XmnI polymorphic site and IVSII.I (G→A) mutation, the results of present study can be used for prediction of the type of β-thalassemia mutation in those with the XmnI site. Furthermore, these findings could help to the manage β-thalassemia intermedia patients to reduce the severity of clinical symptoms.

p-607
A SINGLE NUCLEOTIDE POLYMORPHISM IN THE MMP-9 PROMOTER AFFECTS TUMOR PROGRESSION AND INVASIVE PHENOTYPE OF BREAST CANCER

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Matrix metalloproteinase 9 (MMP-9, gelatinase-B, collagenase type IV-B) is a proteinase capable of degrading a broad range of extra cellular matrix components and basement membrane compounds. In addition to its role in physiological tissue remodeling, MMP-9 has a potential role in some cancers specially, in breast and colorectal. The regulation of MMP-9 gene (MMP9, chromosomal location 20q13.2) expression is not definitely known, but there is evidence that the promoter region polymorphism of MMP9 partly explains the heterogeneity in MMP-9 transcription. The aim of this study was to determine the effect of this polymorphism in breast cancer progression and invasion of patient in Isfahan .The level of MMP9 expression can be influenced by single nucleotide polymorphism (SNP) in the promoter region of this gene. This SNP is located at position 1562 bp upstream of the transcriptional initiation site. MMP9 SNP has been correlated to the risk of initiation and invasion of several tumors such as renal cell carcinoma and breast cancer. Breast cancer is the most common cancer in Iran and the aim of this study is to evaluate the impact of MMP9 C□T polymorphism on invasion and metastasis of breast cancer. So, the MMP9 SNP was genotyped by polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) analysis in 100 breast cancer patients and 100 control subjects. Breast cancer patients were divided in a group without metastasis (M-) and a group that had developed metastasis (M+). We found higher risk for metastasis progression and invasion in breast cancer for TT allele than CC and CT alleles, be significantly higher among patient in M+ group. So, a simple genetic analysis of this polymorphism may provide a useful and potentially important mechanism for predicting prognosis in breast cancer treatment. In addition, we could select MMP9 as a target for therapeutic strategies in all stage of tumor invasion and metastasis.

O-608

MOLECULAR INVESTIGATION OF THE BUNDLE FORMING PILI (BFP) OF AEROMONAS VERONII BV. SOBRIA ROLE IN COLONISATION AND BIOFILM FORMATION

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Mesophilic Aeromonas are ubiquitous water-borne bacteria that can cause a number of diseases in humans and are able to form biofilms. It is supposed that like many other pathogenic bacteria, the bundle-forming pilus (Bfp), which is predominant pilus type, expressed on A. veronii bv. sobria, could be essential for colonisation. Bfp is thought to be the major adhesin of Aeromonas spp. We believe this appendage is required for the colonisation of host cells and for the attachment to abiotic surfaces to form biofilms. Investigation on this hypothesis was done by isolating the genes of this structure, creating a series of isogenic mutants, testing whether they were essential for the formation of the pilus structure and for adherence to various adhesion models. The genes that encode the pilus structural proteins were isolated by PCR using degenerate primers and isogenic mutants were created by insertion of a Kanamycin cassette within the genes (mshA and mshB) and allelic exchange. The mshA and mshB mutants had reduced adhesion to HEp-2 cells and did not form biofilms as readily as the wild type strains. The complementation analysis was done with the copies of the wild type mshA and mshB genes expressed on plasmids introduced into the defective Aeromonas strains and study the adhesion properties of the bacteria. The collective data suggested that the Bfp plays a master role in Aeromonas adherence.

p-609

META-ANALYSIS OF THE MOST COMMON MUTATIONS IN CONNEXIN 26 GENE

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Background: Connexin26 gene, which forms plasma membrane channels between cochlear cells, is a major cause of non-syndromic hearing loss in many populations. More than 90 mutations have been described in this gene, but a few mutations are the cause of deafness in the majority of cases in different populations. Methods: We analyzed 35delG carrier frequencies in 11053 random controls and 235delC frequency in 975 random normal subjects from all over the world. We also analyzed and collected here the carrier frequency of the most common mutations (35delG, 167delT, 235delC) in the Cx26 gene in different ethnic groups. Results: Average frequency of carrier was found to be 2.1, 1.9, 1.1, 1 and 0.51 for European, American, Asian, ocean, and African populations, respectively. Conclusions: We found that average of 35delG carrier frequency is highest in Europe and lowest in Africa. This study highlight the importance for establishing prevalence, based on the local population, of specific and common GJB2 mutations for screening of live births.

p-610

IDENTIFICATION AND ANALYSIS OF THE GENES EXPRESSED IN THE EJACULATED SPERMATOZOA

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Progress and completion of spermatogenesis is related to simultaneous expression of different genes. Recent studies show that many genes are expressed in the sperm and several RNA copies are present in the mature sperm. Identification of these genes and evaluation of their functions would improve our understanding of molecular basis of fertilization, early embryo cleavage and the causes of many types of unexplained male infertility. In this study, we investigated the expression

of DAZ, SYCP3 and GAPDHs genes in ejaculated spermatozoa. 15 semen samples were collected from men referring to Avesina infertility clinic. Normal semen samples (according to WHO criteria) were subjected to density-gradient centrifugation to specifically recover the pure fraction of motile spermatozoa with normal morphology. Total RNA was extracted from the sperm pellets and cDNAs were analyzed using RT-PCR. Expression of SYCP3 and GAPDHs (testis specific genes) was evaluated by nested PCR. The cDNA synthesized from normal testis tissue was used as positive control. Study on cDNAs showed that DAZ and GAPDHs genes are expressed in normal human testis and all evaluated mature spermatozoa samples. According to our previous study the expression of SYCP3 is started from spermatocyte level in human testis during spermatogenesis. However, we did not found any transcript of this gene in spermatozoa. It is estimated that mRNAs of the testis specific genes, GAPDHs and DAZ, may participate in later sperm functions such as fertilization and early embryo cleavage. Detection of such transcripts in sperm will help us understand the molecular basis of fertilization.

p-611
IDENTIFICATION OF ANTI-LISTERIAL GENE, ENTEROCIN A, IN TRADITIONAL IRANIAN LIGHVAN CHEESE

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One of the most popular traditional cheeses in Iran is the semi-hard cheese named Lighvan that is made from raw ewe's or goat's milk without the addition of a selected starter culture. Due to its excellent flavor this kind of cheese is highly preferred by Iranian consumers. The micro flora of Lighvan cheese is dominated by lactic acid bacteria. Enterococci make up one of the most common group of microorganisms in this cheese. In this research, all Enterococcus isolates were identified as E. faecium. Using specific primers for the structural enterocin A gene, the bacteriocin structural gene was found in 21 isolates out of 30 selected isolates. Enterocin A produced by E. faecium strains displayed an antimicrobial spectrum mainly directed against Listeria monocytogenes. The presence of this gene in the wild E. faecium indicates that they might be used as industrial cultures to guarantee the safety of traditional characteristics of this kind of products.

p-612
Y184C MISSENSE MUTATION IN TACSTD2 IN AN IRANIAN GELATINOUS DROP-LIKE CORNEAL DYSTROPHY (GDLD) PEDIGREE

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GDLD is an autosomal recessive ocular genetic disorder characterized by the deposition of amyloid material in the subepithelial and superficial space of the cornea. TACSTD2 (Tumor Associated Calcium Signal Transducer 2) has been identified as the major gene responsible for GDLD. The disease has highest prevalence in Japan, but has also been reported in other countries including India, Tunisia, China, Turkey, and Estonia. We have recently reported that E227K in TACSTD2 is a founder mutation in Iran and that most Iranian GDLD patients carry this mutation. Most Iranian GDLD patients are inhabitants of Northern Iran. We now report results of TACSTD2 mutation screening in an Iranian GDLD pedigree from western Iran. The proband and all other affected members carried the mutation C.551A>G in TACSTD2 in the homozygous state. Most of unaffected members carried one copy of the mutation. C.551A>G causes substitution of tyrosine by cysteine at position 184 (p.Y184C), which lies between the thyroglobulin and transmembrane domains of TACSTD2. Tyrosine is a highly conserved residue at that position. Y184C has previously been reported as a disease associated variation in a Chinese GDLD patient. An intragenic SNP haplotype associated with the mutation in the Iranian patient was identified. Since corresponding information from the Chinese patients is not available it could not be assessed weather the two mutations have a common origin.

p-613
SCREENING OF BETA GLOBIN GENE MUTATION IN BETA THALASSEMIA MAJOR PATIENTS IN KHUZESTAN PROVINCE

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Background: Considering the vast spectrum of beta thalassemia mutations beta thalassemia in Iran, which has a multiethnic population, is the most common hereditary disorder. Methods: after DNA extraction, ARMS-PCR was applied in parallel with direct sequencing of beta globin gene to screen for all mutations in 202 beta thalassemia major patients in Khuzestan province. Results: we found 28 beta thalassemia allelic mutations in 404 alleles studied. Our results show that IVSII-1(G>A) with a frequency of 21.3% (86/404 alleles) represented the most common mutation followed by the four mutations, IVSI-110 (G>A) (17.8%), CD36/37 (-T) (16%), IVSI-5 (G>C) (6.9%) and CD5 (-CT) (5.2%). We found rare mutations including: CD6 (-A), CD31 (-C), CD31 (-C), IVSI-2 (T>G), cd41 (-C), CD50 (ACT>CCT) each with a frequency of 0.25% (1/404 alleles). Conclusion: our findings indicate that Khuzestan population has a wide variety of thalassemia allelic distribution. It is necessary to determine the frequency and distribution of mutations in the different parts of Iran. These results can be used as a basis of prenatal diagnosis of beta thalassemia.

p-614
STUDY OF PHYLOGENIC RELATION IN DIFFERENT POPULATION OF ARTEMIA PARTHENOGENETICA IN IRAN

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Artemia, a halophilic branchiopod genus, is regarded as a complex of sexual and parthenogenetic forms (parthenogenetic populations grouped under the binomen Artemia parthenogenetica). We analyzed 16 S rRNA RFLP marker from seven different parthenogenetic populations of Iran: Shoor and Inche-Borun lakes in Golestan, Maharloo and Bakhteghan lakes in Fars, Namak and Hoze-Soltan lakes in Qom and Arak's Mighan pool in Markazi, in comparison with each other and with bisexual references sample, A. urmiana and A. franciscana. The results indicated significant intra and inter specific divergences as well as pronounced diversity. Maximum parsimony (by PAUP) was largely congruent in reconstructing the phylogeny of the samples. The 16S rRNA data revealed that in spite of some differences in position of samples between phylogenic trees, strict and N.J distance tree, Iranian A. parthenogenetica clustered into two well supported clades: 1- Shoor, Inche-Borun, Namak, Hoze-Soltan and some samples of Maharloo 2- Mighan, Bakhteghan and other samples of Maharloo. Positions of samples in comparison with references samples were not similar. The second clade was grouped with A. franciscana while the first clade was near to A. urmiana. The results compared with similar study on phylogenic relation of bisexual and parthenogenetic species, indicated the technique can be used for taxonomic classification of different populations of A. parthenogenetica in Iran.

p-615
GENETIC IDENTIFICATION OF ARTEMIA PARTHENOGENETICA FROM VARIOUS ECOLOGICAL POPULATION OF IRAN USING PCR-RFLP

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Considering the importance of genetic study to find inter population differences in various species, this study conducted to examine the mitochondrial molecular genetic marker in a small filter feeding organism, Artemia, a genus regarded as a complex of bisexual and parthenogenetic forms. Parthenogenetic populations grouped under the binomen Artemia parthenogenetica inhabit hypersaline water of seven lakes located in different part ofIran: Shoor and Inche-Borun lakes in Golestan province, Hoze-Soltan and Namak lakes in Qom province, Maharloo and Bakhteghan lakes in Fars province and Arak's Mighan pool in Markazi province. A total of 210 individual specimens were collected and DNA extracted by phenol-chloroform method. The mitochondrial rDNA gene region was amplified using the polymerase chain reaction followed by RFLP analysis based on 10 restriction endonuclease (Alu I, EcoR I, Eco47 I, Hae III, Hind III, Hinf I, Mbo I, Msp I, Rsa I, TaqI). Analysis at the inter population level indicated that the haplotype diversity value varied from 0 in Hoze-Soltan, Namak and Bakhteghan samples to 0.7425 in Inche-Borun and Shoor. Furthermore nucleotide diversity varied from 0 (Hoze-Soltan, Namak and Bakhteghan) to 0.007751 for Mighan. Analysis at the intra population level also indicated higher nucleotide diversity value for combination of Mighan vs. Inche-Borun and Shoor (0.170023) and lower values for combination of Hoze-Soltan vs. Namak (0). Our results clearly distinguished nucleotide divergence (minimum in Inche-Borun vs. Shoor, %-0.205, and maximum Inche-Borun and Shoor vs. Mighan, %16.1879). The population difference based on haplotypes frequency was statistically significant ($p<0.001$) except for Hoze-Soltan vs. Namak and Inche-Borun vs. Shoor. These findings suggest genetic difference in A. parthenogenetica populations from Iran. There are enough evidences in haplotypic level for separating of A. partenogenetica in Iran to five populations: Hoze-Soltan and Namak, Mighan, Maharloo, Bakhteghan, Inche-Borun and Shoor that may be important in understanding the ecology of this commercially important organism.

p-616
STUDYING OF EXON 10 OF CFTR GENE AMONG CYSTIC FIBROSIS PATIENTS FROM NORTH-WEST OF IRAN BY PCR-SSCP METHOD

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Cystic fibrosis (CF) is the most common severe autosomal recessive disorder in Caucasian population and affects 1 in 2500 live births. This disorder is the result of mutations that affect the coding region of CFTR gene and results in a defective flow of Cl^- and Na^+ ions, and high concentrations of these ions in sweat. F508 is the most common CF mutation in all populations to date. The mutation is located in exon 10 of this gene. In this project we studied the exon 10 of this gene between CF patients referred from East Azerbaijan region by applying PCR-SSCP method. The sample analyzed consisted of 46 families with a clinical diagnosis of CF. Twelve different patterns of SSCP were observed among 30 affected families (65%). Twelve patients showed homozygous and 17 patients showed heterozygous pattern for SSCP of exon 10. In one of the families, the affected child was deceased and its parents showed heterozygote pattern for one SSCP pattern. This data suggests that PCR-SSCP together with sequencing

technique can be used in screening of population and for prenatal diagnosis of affected families.

p-617
DEVELOPMENT AND OPTIMIZATION OF A RAPID AND ACCURATE MULTIPLEX-NESTED PCR ARMS FOR GENOTYPING SNP8NRG221533 OF HUMAN NRG1 GENE

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Schizophrenia is a severe neuropsychiatric disorder with symptoms such as hallucination, delusion and thought disorder. It is a complex disorder, which genetic components play a crucial role in its pathogenesis. Among candidate genes for schizophrenia, NRG1 is one of the most significant, which has been confirmed by several studies. SNPs located in 5′ upstream of NRG1 have shown significant association with schizophrenia in several populations. We developed a Multiplex-Nested PCR ARMS for genotyping a single SNP (SNP8NRG221533) in human NRG1 gene. The association of this SNP with schizophrenia has been confirmed in several studies. Notably, no restriction site can be found for distinguishing T and C allele of this SNP. Thus genotyping would be impossible by commonly used restriction enzyme method. 30 healthy and 30 schizophrenic patients were genotyped by this method and results were confirmed by sequencing. The results of Allele and genotype frequencies compared between healthy and affected individuals will be presented in this report.

p-618
INSERTION/DELETION (I/D) GENE POLYMORPHISM OF ANGIOTENSIN CONVERTING ENZYME IN PATIENTS WITH CORONARY ARTERY DISEASE AND IN HEALTHY SUBJECTS FROM IRAN

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The rennin-angitensin system plays an important role in the pathogenesis of vascular disease and angiotensin-converting enzyme (ACE) is the major part of this system. There are several polymorphisms in ACE gene. The insertion/deletion (I/D) polymorphism is the most important one due to its association with cardiovascular disorders. This polymorphism is occurred because of insertion (I) or deletion (D) of a 287 bp Alu sequence, resulting in 3 genotypes; DD, DI and II. It has been reported that the DD genotype may have a relation to cardiovascular disease susceptibility. As coronary artery disease (CAD) and atherosclerosis is the main cause of heart failure, the relevance of ACE polymorphism for CAD was determined in the Iranian population. 487 individuals including 224 patients with >50% angiographically established coronary stenosis and 263 healthy subjects genotyped by a standard method, The results showed that there is no increased risk of CAD in association with DD genotype in Iranian population. Allele frequencies were also similar in both groups. Moreover, systolic and diastolic blood pressure, serum cholesterol and LDL-C were significantly increased in CAD patients. Therefore, DD genotype does not increase the CAD susceptibility in the studied population. Further studies together with the other polymorphisms of ACE gene may be required to determine the relation between cardiovascular disease susceptibility and ACE genetic variations in Iranian population.

p-619
MOLECULAR INVESTIGATION OF PHENYLALANINE HYDROXYLAE GENE MARKERS IN ISFAHAN POPULATION

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Deficieny in phenylalanine hydroxylase (PAH) due to mutations in the PAH coding gene, is the main molecular characteristic of phenylketonuria (PKU) disease. More than 400 different mutations have been reported associated with the disease. However, mutation pattern of the PAH gene in Iranian population has not been established yet. A number of molecular markers, which are highly linked to the gene, could be used in carrier detection and prenatal diagnosis of the disease. Application of the markers is dependent on their degree of heterozygosity and allele frequency, which is not studied in Iranian population. These include several restriction fragment length polymorphic (RFLP) markers as well as an intra-genic short tandem repeat (STR) in intron III, and an inter-genic variable number of tandem repeat (VNTR) at the 3´ end of the gene. Here we report the data on the degree of the heterozygosity and allele frequency of these markers investigated in Iranian population (mainly residing in the province of Isfahan) using PCR/RFLP technique.

p-620
INTERLEUKIN 10 GENE POLYMORPHISM IN BONE MARROW TRANSPLANT RECIPIENTS

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Objective: Graft-versus-host disease (GvHD) is the main complication after hematopoietic stem cells transplantation (HSCT) and occurs even donor (D) and recipient (R) HLA matching, apparently because of D/R minor histocompatibility antigen mismatches and cytokine polymorphisms. IL-10 suppresses several activities of the immune response by inhibition of Th1 and Th2 cells. These properties suggest that

IL-10 could act as a suppressive mediator and prevent GvHD. The current study attempted to evaluate the association between IL-10 promoter gene polymorphism and transplant outcomes among 18 recipients of cytokine-mobilized peripheral blood stem cell (PBSC) from HLA matched sibling donors Method: We analyzed three single-nucleotide polymorphisms in proximal region of IL-10 promoter gene (-1082/-819/-592) by ARMS and PCR-RFLP methods. Eighteen patients and their recipients undergone allogeneic PBSCT at bone marrow transplant center in Nemazi hospital (Shiraz, Iran) between September 2005 to September 2006, were included in this study. Results: The haplotype (1082*G/819*C/592*C [GCC] is predominant in both D and R. But no significant association between GCC haplotype and the risk of acute GvHD was detected in both D and R. (P = 0.56). Conclusion: IL-10 promoter gene polymorphism was not found to be apparently associated with acute GvHD after allogeneic peripheral blood stem cell transplantation from HLA-matched sibling donors. Further studies with larger sample are necessary to define the influence of IL-10 on the immune response after BMT. Key words: Bone marrow transplantation, Graft versus host disease, Interleukin 10, polymorphism.

p-621
DETECTION OF HUMAN HERPESVIRUS 8 DNA IN PEMPHIGUS AND CHRONIC BLISTERING SKIN DISEASES

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Pemphigus is a rare autoimmune blistering disorder of the skin and characterized by circulating IgG antibodies in the serum of affected individuals. In pemphigus, the antibodies mistakenly consider normal tissue as foreign and attack them. This produces painful raw areas on the skin and mucous membranes that will not heal. Before modern drug treatment death from overwhelming infection was the usual outcome but this is no longer common. Increased incidences of Kaposi's sarcoma and lymphoid malignancies have been observed in patients with pemphigus and the human herpesvirus 8 (HHV8) is very strongly associated with this tumors. Because the virus may be one of the triggering factors of pemphigus , we undertook this study to screen for the presence of HHV8 in chronic blistering skin diseases including pemphigus. DNA specimens were extracted from paraffin-embedded tissues. The samples were deparaffinized using iso-octan and absolute ethanol. Then, the tissue pellets were digested in lysis buffer (proteinase K 20 mg/ml tris HCL 50mM EDTA 1mM tween 0.5%). A total of 62 paraffin-embedded specimens were studied using nested polymerase chain reaction (Nested-PCR) with specific primers to amplify a 138- base pair HHV8 fragment. HHV8 DNA was detected in 15 of 42 (35.7%) specimens from pemphigus but not in 20 normal skins of individuals obtained by cosmetic surgery. The results of our study suggested that HHV8 infection might have tropism for pemphigus lesions and might be a contributing factor in the development of pemphigus.

p-622
STUDY OF INOSINE TRIPHOSPHATE PYROPHOPHATASE GENE EXPRESSION IN CHRONIC MYELOGENOUS LEUKEMIA (CML) PATIENTS

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Genetic material in nucleus or mitochondria is the major intracellular target of damages that cells are exposed every day. The presence and accumulation of these damages can lead to genetic instability and mutagenesis, which is the perquisite of different types of diseases or cancers. The accurate repair of genetic material for normal function of eukaryotic genome relies on the presence of a faithful enzymatic repair system. One of the most significant damages is the incorporation of oxidative deaminated nucleotides such as inosine triphosphate (ITP, dITP) into DNA or RNA. It has been suggested that inosine triphosphate pyrophosphates (ITPase) encoded by ITPA gene, has the main role for protecting cells against oxidative deaminated nucleotides by hydrolyzing them to their monophosphate counterpart. Several evidences support the harmful role of ITP and dITP in genetic and chromosomal instability. Therefore, we suppose that dysfunction of ITPA gene might be an important factor as genetic and predisposing background to diseases or malignancies. Chronic myelogenous leukemia (CML) is a type of leukemia, which is mainly caused by Philadelphia chromosome. There are some reports about existence of several structural and numerical chromosome abnormalities in addition to Philadelphia chromosome in these patients. To examine the possible involvement of ITPA dysfunction in CML etiology, we studied the ITPA gene expression in blood samples collected from CML patients by semi-quantitative RT-PCR and will present our results in this report.

p-623
BUTYRYLCHOLINESTERASE K VARIANTS INCREASE THE RISK OF CORONARY ARTERY DISEASE IN THE POPULATION OF WESTERN IRAN

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Several conflicting studies has suggested an association between Butyrylcholinesterase-K variant (BCHE-K,G1615A /Ala539Thr) and the risk of developing coronary artery disease (CAD) in diabetes and non-diabetic subjects. The objective of this study was to determine the association between BCHE-K variant and the risk of CAD in patients with

and without Type 2 diabetes mellitus (T2DM), from western Iran. This case–control study consisted of 464 subjects, who underwent their first coronary angiography. They were matched and randomly assigned into four groups: CAD+T2DM+ (CAD/T2DM), CAD+DM- (CAD/ND), CAD−DM+ (T2DM/NCAD) and CAD−DM− (control). BCHE-K variant was detected by PCR-RFLP. The BCHE-K allele frequency in CAD patients with and without T2DM [total CAD (TCAD)] and separately in each group (CAD/T2DM and CAD/ND) were significantly higher than in control group (21.1%versus13.3%; p=0.001, 22.4%versus13.3%; p=0.001 and 19.7% versus13.3%; p=0.015, respectively). The OR for the BCHE-K heterozygous and homozygous in TCAD subjects were 1.65 (95%CI 1.17-2.3, p=0.004) and 4.3 (1.05-19.4, p=0.048), CAD/T2DM individuals were 1.76 (1.2-2.6, p=0.004) and 4.73 (0.96-23.3,p=0.052) and for the CAD/ND patients were 1.53 (1.05-2.3,p=0.029) and 3.88 (0.8-19.7,p=0.7), respectively. OR of BCHE-K allele was found to be 1.74 (1.1-2.4, p=0.001) in TCAD subjects, 1.87 (1.12-1.48, p=0.001) in CAD/T2DM group and 1.59 (1.04-1.4,p=0.016) in CAD/ND subjects. These data suggest that the BCHE-K allele increases the risk of CAD in the population (with and without DM) in western part of Iran and its presence intensifies the risk of CAD in the T2DM. The fact that the BCHE-K, even in the heterozygous form, exacerbates the risk of CAD in this population, suggests that a specific therapeutic intervention should be considered for this particular group of patients.

p-624
COMPARISON OF THREE METHODS OF DNA EXTRACTION FROM PARAFFIN-EMBEDDED TISSUES

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Paraffin-embedded tissues are valuable resources for molecular genetic studies but the extraction of high quality nucleic acid from them may be problematic. Most DNA extraction methods consist of three stages: (1) deparaffinization; (2) protein digestion; and (3) DNA purification. In this study three different techniques were used for optimizing the DNA extraction from paraffin-embedded tissues in order to yield to suitable DNA for PCR. In all these techniques the deparaffinization stage was similar and it was performed by dissolving the paraffin using xylene and ethanol. DNA extraction methods are: (1) modified phenol-chloroform protocol; (2) salting out method using Ammonium acetate; and (3) commercial kit (QIA amp DNA Mini Kit (50)-QIAGEN). DNA was qualified and quantified using spectrophotometer analysis, electrophoresis, and PCR amplification. The tissue specimen used for detecting p53 and nat2 genes consisted of liver tissues with hepatocellular carcinoma (HCC) and stomach tissues, which showed stomach adenocarcinoma. The 196 bp and 780 bp fragments of p53 and nat2 genes, respectively, were amplified using PCR after DNA was extracting by the means of the mentioned methods. Finally, comparing the electrophoresis pattern in all three methods, no significant differences were detected and all three methods showed equally good results. The results indicated that although the phenol-chloroform method is effective for extracting high quality DNA but the toxic solutions used and its time consummation reduces its benefits. However salting out method was more convenient and less toxic than the phenol-chloroform method. Thus, DNA extraction using salting out method in similar situations is recommended.

p-625
PHOSPHOLIPID HYDROPEROXIDE GLUTATHIONE PEROXIDASE GENE POLYMORPHISM IN IRANIAN INFERTILE MEN

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In this study we analyzed the relation between sperm DNA damage by oxidative stress and sexual hormones production. We also studied the relationship of sperm DNA damage and the amount of sperm nuclear protamine. We also investigated polymorphisms of gpx-4 gene in Iranian infertile men using PCR-RFLP method. The study of gpx-4 gene polymorphism was conducted on 128 men including 74 infertile men with abnormal spermogram, 18 normospermic men and 36 fertile men as controls. For oxidative stress study133 men, 82 men with abnormal sperm parameters and 52 normozoospermia were included. The gpx-4 gene with the nucleotide sequence of +6 (C-*T), +17 (G-»A) and +1725 (G-»A) were analyzed using PCR-RFLP.MDA and TAG was measured by TBA assay and Randox kit using spectrophotometer in seminal plasma, respectively. Digestion of a 237 bp intact PCR product by Mwol generates two fragments (151 bp and 86 bp). When a mutation occurs in the restriction site +6 (C-»T), the enzyme would not recognize the sequence, therefore 237 bp segment remains undigested. Treatment of 237 bp segment with PshAI generates two fragments (161 bp and 76) in the intact gene but the same enzyme cannot digest 237 bp segment when a mutation occurs in the restriction site +17 (G-»A). Finally, digestion of 148 bp intact fragment with SatI generates two fragments (108 bp and 40 bp) but when a mutation occurs in the restriction site +1725 (G-»A), the enzyme will not recognize the sequence. Therefore, the148 bp fragment remains undigested. Evaluation of enzymatic digest of 237 bp and 148 bp segments in all participants revealed that none of the investigated mutations is present in GPX-4 gene. Based on our findings the prevalence of these mutations in Iranian infertile men is probably low and there might be no association with the etiology of the disorder affecting sperm parameters. Therefore, to determine the exact prevalence of these and other mutations of the gene in Iranian infertile men a study with a larger number of patients is suggested.

p-626
THE CORRELATION BETWEEN HELICOBACTER PYLORI INFECTION AND GASTRIC CARCINOGENESIS IN IRAN

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Gastric cancer is the fourth most common cancer and the second most common cause of cancer-related death worldwide. Helicobacter pylori, the group I carcinogen for humans, plays a specific role in the development of atrophic gastritis, which represents the most recognized pathway in multi-step intestinal-type gastric carcinogenesis. Helicobacter pylori strains possessing the cag pathogenicity island (PAI) have been associated with increased gastric inflammation. Recent works have shown that there is a close correlation (about 80% H.pylori+) between H.pylori infection and gastric cancer. In contrast; studies on the association of cagA H. pylori infections and gastric cancer in Western countries and East Asian countries have shown conflicting results. To determine the correlation between H.pylori infection and gastric cancer and also the cagA status in Iran, we investigated 30 formalin fixed paraffin embedded tissue sections of thirty different patients suffering gastric carcinogenesis. We performed DNA extraction and polymerase chain reaction (PCR) amplification using specific set of primers for phosphosamine mutase (glmM) gene to detect H.pylori strains and the conserved region of cagA gene to analyze cagA status. H.pylori was detected in 76.66 percent (n=23) of patients with gastric cancer, whereas only 13.04% (n=3) of them were positive for cagA gene, suggesting that although H.pylori infection is significantly associated with gastric cancer in Iran, but CagA is not the dominant virulence factor in inducing gastric carcinogenesis.

O-627
MOLECULAR DIAGNOSIS OF CHRONIC GRANULOMATOUS DISEASE IN IRAN

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Chronic Granulomatous Disease (CGD) is an inherited phagocytic disorder caused by mutations in NADPH oxidase subunits. Patients with CGD suffer life-threatening bacterial and fungal infections. During two years of study forty-five families with clinically diagnosed CGD were referred to Children's Medical Center at Tehran University as the referral center for immunodeficiency in Iran. Neutrophil functional assays performed for affected children and their mothers showed no activity or residual activity in affected neutrophils. PMN (Polymorphonuclear leukocytes) oxidative burst revealed mosaic pattern in 12 mothers. Western blot analysis revealed X91 phenotype in all their sons. Mutation screening in CYBB gene using SSCP analysis followed by sequencing, showed 9 different mutations including one novel mutation. Western immunoblot subtyping of patients whose mother showed no mosaic pattern by DHR123 revealed 24 patients with p47 null expression, 7 and 2 patients with p22 and p67 defect, respectively. ΔGT screening in Ncf1 gene for p47 patients, revealed 8 patients with this mutation. Mutation analysis for the rest of Ncf1 gene in these patients is understudy. CYBA mutation analysis revealed 6 different mutations including five novel mutations in p22º patients. Overall, the number of autosomal recessive patients with CGD in Iran is high. It seems consanguineous marriages might be one of its causative factors.

p-628
THERMOPHILIC MICROORGANISMS AND THERMOSTABLE ENZYME FROM JAVA HOT SPRINGS

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Biocatalyst industry using ordinary enzymes to extremozyme explored thermophilic microorganisms. Indonesia as one of a tropical country surrounded by very tectonically active region is best place for habitat of thermophiles microorganisms. Biodiversity of thermophilic microorganisms from a few hot springs located throughout Java were studied by analyzing 16S rRNA. The results showed that most of microorganisms found were uncultivable bacteria and archeabacteria. A few of them were identified as new strains, probably new genus. Some of these microorganisms were successfully cultivated as thermophiles. DNA polymerase gene from one of these organisms was cloned and sequenced. Cloning was carried out by Polymerase Chain Reaction (PCR) strategy. Two sets of primers, internal and external primers, were used to amplify the whole coding region of the gene. Sequence comparison to database proved that the gene was belonging to DNA POL I family, with the highest homology to DNA POL I from Bacillus caldolyticus. The gene was successfully expressed in E. coli through expression vector pTRXFus. The crude extract and partial purified of the fusion protein still showed high polymerase activity and thermostability with the optimum activity at pH 7.4 and 65º C.

p-629
T1, M1 AND P1 POLYMORPHISM OF GLUTATHIONE S-TRANSFERASE GENE AND DIABETES MELLITUS RISK IN KERMANSHAH

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Oxidative stress is associated with damage to lipids, proteins and nucleic acids, which leads to the pathophisiology of various diseases including diabetes mellitus. Glutathione S-transferases (GSTs) are members of multigenic family, which have essential role in the cell as antioxidant agents. To determine the possible relation between polymorphism of these genes and development of diabetes mellitus, polymorphism of these enzymes has been investigated in different diseases. In the present study, we investigated, the polymorphism of GSTT1, M1 and P1 genotypes in diabetic (n=76) patients compared to controls (n=74). The case-control study was conducted on 150 subjects. The GSTT1, M1 and P1

genotypes were determined by polymerase chain reaction and restriction fragment length polymorphism procedure. Our results revealed that in diabetic patients compared to controls, the frequencies of GSTT1-Null and GSTM1-Null genotypes were significantly decreased ($p=0.004$ and $p<0.001$). However, considering GSTP1 genotypes, there was no significant difference between groups ($p=0.865$). The results suggest that, decreased frequencies of GSTM1-Null and GSTT1-Null genotypes could be associated with an increased incidence and development of diabetes mellitus.

p-630
THE FIRST REPORT ON MUTATIONS OF EXONS 14-17 FROM INSULIN RECEPTOR GENE IN IRANIAN PATIENTS WITH TYPE II DIABETES MELLITUS

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Diabetes mellitus is a complicated metabolic disease, recognized by constant hyperglycemia. Diabetes occurs when the body can't produce insulin (type I), or is not able to use the produced insulin (Type II). There are some reports of mutations in insulin receptor gene, which leads to insulin resistance. The aim of this research was determining mutations of exons 14, 15, 16, 17 of insulin receptor gene (tyrosine kinase domain) in Iranian patients with type II diabetes. We extracted DNA of 128 blood samples from patients with NIDDM and amplified exons 14, 15, 16 and 17 by specific primers and PCR products were sequenced. We detected some mutations in insulin receptor gene of Iranian patient with NIDDM. In exon 14, the C at position 2706 was replaced by G (Arg 902 Arg) and C at position 2717 was replaced by G (Ala 906 Gly). A missense mutation was found in exon 14; C at positions 2752 and 2753 (CCG --- TGG) was replaced with T (Pro 918 Trp). A heterozygote missense mutation was found in exon 17. Therefore, T at position 3257 was replaced by A in codon 1086 (Val 1086 Gln). We didn't detect any mutations in exons 15 and 16. The mutations observed in insulin receptor gene of Iranian patients with type II diabetes is different from the ones reported from other countries. Further studies are required to confirm these results.

p-631
USING MULTIPLEX-NESTED-RT-PCR FOR QUICK CHARACTERIZATION OF COMMON TRANSLOCATIONS IN CHILDREN LEUKEMIA

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Acute lymphoblastic leukemia (ALL), the most common subtype of childhood cancer, is a heterogeneous disease that is associated with the presence of specific chromosomal translocations. Detection of specific chromosomal translocations allows the identification of prognostically relevant subgroups. Molecular-based assays can be performed successfully on a higher proportion of cases than conventional karyotyping. Although FISH techniques are equally capable to detect these fusion transcripts, performing these tests on indivial samples is not very efficient. Our aim is to develop a highly sensitive and specific method to screen simultaneously for the four most frequent translocations in ALL: t (9; 22), t (1; 19), t (4; 11), and t (12; 21). Multiplex RT-PCR assay is an effective, sensitive, accurate and cost-effective diagnostic tool which can improve the ability to stratify accurately and rapidly patients with childhood ALL.

p-632
ABCB1 GENE POLYMORPHISM AND ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

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Introduction: P- glycoprotein (Pgp) is a transporter pomp coded by ABCB genes family and protects the cell from xenobiotics and toxic compound. Polymorphism of these genes can influence on the risk of developing diseases specially cancers. Recently a single nucleotide polymorphism, C3435T of ABCB1gene, has been found to be associated with altered tissue expression of Pgp. We tried to evaluate C3435T ABCB1 gene polymorphism in patient with ALL. Materials and methods: 130 patients with ALL and 139 healthy persons as a control have been investigated in this study. We used PCR-RFLP, polymerase chain reaction- restriction fragment length polymorphism methods to compare the incidence of ALL between two groups. Results: Chi - square analysis showed that the mutant homozygous TT genotype was associated with occurrence of ALL (OR=1.96 CI=1.07-3.58; p-value=0.026). Conclusion: Incidence of ALL is more in individuals with TT genotype of C3435T ABCB1 gene than other carriers.

p-633
A MOLECULAR APPROACH FOR EFFICIENT EXPRESSION OF HUMAN FACTOR VIII IN MAMMALIAN CELLS

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A current treatment for the X-linked haemophilia a disease, infusion of plasma-derived human factor VIII (hFVIII), has the risk of transmission of blood-borne viral agents. Therefore using recombinant DNA-derived FVIII make haemophilia A patients independent of blood-derived products. Therefore efficient system for over-expression of recombinant hFVIII

(rhFVIII) is required. It has been shown that the inclusion of introns increase gene expression. Therefore, to enhance the expression level of hFVIII in mammalian cells we consideredintroducing of an intron. The effect of intron-1 of human factor FIX (hFIX) gene to increase the expression level of corresponding proteins was shown previously. Therefore in the present work a hFVIII expression plasmid containing a truncated intron-1 of the hFIX in the 5' end of hFVIII reading frame was constructed. The intron-containing hFVIII expressing plasmid was used to transfect CHO cells and several recombinant colonies were isolated and subjected for expression analysis. Expression of hFVIII was indicated by performing one-stage clotting assay on the media collected from the transfected cells. Comparison of the clotting time of the media from the intron–containing clones with the original hFVIII expressing plasmid at the same conditions showed an elevated level of hFVIII in the clone with the hFIX intron-1. Further quantitative expression analysis on the clones with the heterologous intron is required to confirm the positive effect of truncated hFIX intron-1 on the expression of hFVIII. This study has provided tools for further molecular studies and engineering of factors influencing the expression efficiency of rhFVIII.

O-634
THROMBOPHILIC MUTATIONS AMONG SOUTHERN IRANIAN PATIENTS WITH SICKLE CELL DISEASE: HIGH PREVALENCE OF FACTOR V LEIDEN

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A hypercoagulable state in sickle cell disease (SCD) and beta thalassemia has been shown previously. A case-control study was performed to determine the prevalence of Factor V Leiden and prothrombin G20210A mutations among sickle cell disease patients from Southern Iran compared to healthy individuals. Patients comprised 60 SCD patients including 35 SS (21 males and 14 females) aged 17.2± 8.3, 15 AS (9 males and 6 females) aged 30 ±15.4 and 10 S/Thal (3 males and 7 females) aged 24.6±10.4. Control group was 126 apparently healthy individuals (50 males and 76 females) aged 20.1 ±9.8. Genotyping was done by PCR-RFLP using Mnl I and Hind III for factor V Leiden and prothrombin G20210A, respectively. A significantly high prevalence of factor V Leiden was found in sickle cell disease patients from Southern Iran compared to normal individuals (around 8 times). Heterozygous factor V Leiden mutation was found in 5 of 35 (14.3%) SS patients, 2 of 15 (13.3%) as individuals, 1 of 10 S/Thal patients (10%) and 2 of 126 (1.6%) control subjects ($P<0.05$). However, only one AS individual (6.7%) was found to be carrier for prothrombin G20210A compared to 5 out of 126 (4%) healthy individuals. Adjusted logistic regression analyses (OR) for factor V Leiden heterozygous in SS patients and AS individuals were 10.3 (95% CI 1.9-55.9, $P=0.007$) and 9.5 (95% CI 1.2-73.5, $P=0.03$), respectively. These findings suggest that the factor V Leiden may contribute to the clinical complications of sickle cell patients.

p-635
IDENTIFICATION AND CHARACTERIZATION OF SHORT TANDEM REPEAT (STR) MARKERS IN HLA-DRB1 REGION

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The major histocampatibility complex (MHC) region, in particular HLA-DRB1 and HLA-DQB genes, has been reported to be associated with susceptibility to multiple sclerosis (MS). However, it has not been elucidated whether the HLA-DRB1 or the nearby genes are responsible for the susceptibility to MS. Therefore, molecular markers in the region could facilitate analysis of the involvement of this region to the pathogenesis of MS. The aim of this study was to identify a number of genetic markers with high allele frequency and heterozygocity in the HLA-DRB1 region. Of the markers in this region D6S2806, M2-3-22, and D6S2879 were analyzed in this study. The markers were analyzed using PCR amplification with specific primers followed by polyacrylamide gel electrophoresis (PAGE) and silver staining. Among the markers tested M2-3-22 failed to produce any PCR product, however, primers for D6S2806 and D6S2879 resulted in the expected-size bands with different length and frequency. These data could be used for further evaluation of the association of HLA-DRB1 locus with MS.

p-636
STUDY OF PERIPLASMIC EXPRESSION OF HUMAN GROWTH HORMONE USING SYNTHETIC SIGNAL PEPTIDES DESIGNED BASED ON TAT AND SEC SECRETION PATHWAY

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The Twine Argenine Translocation pathway (Tat-pathway) is an export system that functions in bacteria in parallel with the SEC-pathway. In this work the potential of the Tat-based signal peptides, were examined for the secretion of human growth hormone, which forms inclusion bodies in the host cytoplasm. With this aim, two Tat-based signal peptides with 3 major domains of typical signal peptides including N-terminal hydrophobic and C-terminal domains were designed in which the N-termial-hydrophobic borders contain two argenine residues in a conserved motif. For translation efficiency, the use of host major codon-usage profile and the secondary structure of the precursor mRNA of the pre-protein was considered. The structure of the signal peptides and their processing efficiencies were predicted with the aid of a neural network-based program. Based on this analysis the signal peptides were designed, to be recognizable either by Tat secretion pathway (Tat-ab) or by both SEC and Tat pathways (Tat-sec-ab). The fragments were constructed by oligonucleotide annealing and cloned in T7-based expression plasmids, followed by sub-cloning of human growth hormone cDNA. The recombinant plasmids were used for the expression analysis in Escherichia coli. The protein patterns of the IPTG-induced recombinant bacteria indicate in the over-

expression of hGH precursors for both of the signal peptides. However the processing of the expressed recombinant protein takes place only in the case of the clone with the Tat-sec-ab signal peptide. Further expression analysis for optimization of growth and induction on the obtained clone is required to achieve a highest expression efficiency hGH in the periplasmic space of E. coli.

p-637
EFFECT OF CHOLESTERYL ESTER TRANSFER PROTEIN TAQ 1B POLYMORPHISM ON LIPID LEVELS AND CETP ACTIVITY IN IRANIAN POPULATION

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Primary hyperlipidemia, characterized by hypertriglyceridemia and/or hypercholesterolemia, is considered to be one of the most important risk factors for atherosclerosis and coronary heart disease. Cholesteryl ester transfer protein gene polymorphisms are known to be associated with changes in lipid levels. We used polymerase chain reaction and restriction fragment length polymorphism analysis to determine the relationship between Taq1B polymorphism and lipid profile. We measured lipids and cholesteryl ester transfer protein activity in primary hyperlipidemic and normolipidemic subjects, with and without Taq1B polymorphism. Genotype distribution and allelic frequencies of polymorphism were determined and compared in both groups. Our results showed that plasma cholesteryl ester transfer protein activity was significantly higher in primary hyperlipidemia than in controls (P=0.001). Plasma lipids were also remarkably higher in primary hyperlipidemic subjects. In both patient and control groups, individuals with B2B2 genotype had lower plasma cholesteryl ester transfer protein activity, higher total cholesterol, higher high-density lipoprotein cholesterol and lower triglyceride than those with B1B1 and B1B2 genotypes. The values of low-density lipoprotein cholesterol were significantly increased in primary hyperlipidemic patients with B2B2 genotype. The genotype and allelic frequencies for this polymorphism differed significantly between primary hyperlipidemic patients and controls (P=0.022 and P=0.039, respectively). Taq1B polymorphism of cholesteryl ester transfer protein gene was associated with changes in lipids profile and plasma cholesteryl ester transfer protein activity in the selected population.

p-638
USE OF HUMAN BETA-GLOBIN GENE INTRON-2 TO ENHANCE THE EXPRESSION OF HUMAN FACTOR IX IN CULTURED MAMMALIAN CELLS

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Hemophilia B, a coagulation-disorder, which is the result of lack of active factor IX (FIX) commonly treated with replacement therapy. Because of the possible transmission of blood-borne pathogens with plasma-derived materials, application of recombinant FIX is preferred. For production of FIX a mammalian expression system is required to provide the necessary protein post translational modifications. Although cDNA constructs without intron can be expressed efficiently in mammalian cells, it has been shown that the inclusion of introns increases its expression level. Some sequences such as beta-globin cDNA, show requirement for the presence of an intron for their expressions. A bioinformatic analysis on the beta-globin gene intron-2 has detected two 96 base pair short interspersed nuclear elements (SINEs) in addition to a number of sequences, which are candidates for transcription factor binding sites. This finding supports a possible regulatory function for this intron. To examine the role of beta-globin gene intron-2, a CMV-regulated hFIX expressing plasmid was constructed, carrying this intron in the corresponding site of hFIX gene. Chinese Hamster Ovary cell was transfected with the recombinant plasmid and grown in selective media. To evaluate the effect of beta-globin intron-2, the transected cells will be subject for the expression analysis in comparison with an intron-less hFIX expressing cells. This work together with the results from the work on intron-1 (presented in parallel) will provide evidence for potential effect of beta-globin introns to enhance the expression level of hFIX. This system could be used for the overproduction of other complex eukaryotic proteins as well.

p-639
ANALYSIS OF CAGA GENOTYPE AND VARIANTS OF THE 3' REGION OF THE CAGA GENE IN IRANIAN HELICOBACTER PYLORI STRAINS AND RELATIONSHIP TO GASTRODUODENAL DISEASES

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Helicobacter Pylori is one of the most common infections worldwide that is associated with gastric inflammation, peptic ulcer and a risk factor for gastric cancer. The clinical outcome of H. pylori infection might be related to the bacterial cagA genotype. The 3' region of cagA gene contains repeated sequences. To investigate cagA genotype and variants in Iranian H. pylori strains and explore their relationship with gastroduodenal disease, the cagA status of 77 Iranian H. pylori strains was examined and variation in size of the 3' region of cagA in all was analyzed by PCR. The consensus region of CagA was identified in 52 (68%) of these strains. CagA was present in 28 (80%) of 35 strains separated from duodenal ulcers, 10 (77%) of 13 strains from gastric ulcers and 14 (48%) of 29 from gastritis. CagA was associated with duodenal ulcer disease (P=0.0078) but not with gastric ulcer

(P =0.08) when compared to patients with gastritis. The variable region analyzed by PCR method was able to detect 31 (42%) cagA positive cases within the same group of H. pylori-positive patients. 23 strains yielded PCR products of 642 to 651 bp, 8 strains had products of 756 bp. These products could be classified into two subtypes of cagA (A, B/D) depending on the type and number of repeats. These subgenotypes were not associated with clinical outcome (P =0.49). CagA was a marker of H. pylori strains for duodenal ulcer disease in the population but the subgenotypes did not show a significant correlation with specific gastroduodenal disease in this study.

p-640
DETECTION OF HTLV1 DNA AND HCV RNA IN NON-HODGKIN LYMPHOMA

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Non-hodgkins lymphoma is a lymphoproliferative neoplasm, which affects T and B-lymphocytes, although the etiology is multifactorial, but the role of hepatitis C virus (HCV) and HTLV1 in the pathogenesis of Non-hodgkin lymphoma (NHL) is controversial. In this study, we detected HCV RNA and HTLV1 proviral DNA in paraffin-embedded tissue from NHLs by RT-PCR and PCR methods. Paraffin- embedded lymphoma tissues were deparafinized using iso-octan and absolute ethanol and then digested in lysis buffer (proteinase K 20mg/ml, tris HCL 50mM, EDTA 1mM, tween0.5%) overnight at 37 C then RNA specimens were extracted using TRIZOL. Random hexamer primers were used for cDNA synthesis by Revert Aid TM M-Mulv enzyme. HCV specific primers amplified 207 bp fragments of HCV core region. To detect HTLV1 DNA, after deparafinizing specimens, DNA extracted using proteinase K and amplification 158 bp and 451bp fragments was performed by specific primers for Tax and LTR regions of HTLV1. Results: HCV RNA was detected in 4 of 52 (7.8%) specimens and HTLV1 DNA was detected in 15 of 52 (29%) specimens from NHL. conclusion: This study suggested that RT-PCR on paraffin-embedded lymphoma tissues is an alternative method of testing for HCV and there is an association between HCV and HTLV1 infections and non-hodgkin lymphoma.

O-641
PRENATAL DIAGNOSIS OF β- THALASSEMIA ON 4 TWIN PREGNANCIES

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β-thalassemia is the most common hereditary disease and is characterized by reduced or absence of β-globin chain synthesis. β -thalassemia causes anemia with different severities ranging from asymptomatic carrier state to transfusion -dependent thalassemia major. Premarital screening program for β -thalassemia prevention has been established ftrom 1997 nationwide and prenatal diagnosis (PND) has been lowered the birth rate of disease dramatically. Among couples referred to our lab for prenatal diagnosis, four mothers had twin pregnancy. Chorionic villus sampels (CVS) were obtained by transabdominal processing at 10-12 weeks of gestation. CVS were dissected under the microscope. DNA was extracted from CVS using standard technique. After characterizing parental mutations by ARMS PCR and performing linkage analysis by polymorphic markers (RFLP), prenatal diagnosis was performed. When the results of mutation and RFLP marker in the fetus and mother are similar, a variable number of tandem repeat (VNTR) markers (PKU, APOB) were used to aviod mother contamination. In two families, the twins were one normal, one minor. For the other family, both of twins were minor, and for the last family, one of twins was major and the other was minor. It is very important for surgeon to know exact position of fetuses, which usually identified by placenta position by radiologist.

p-642
HAPTOGLOBIN PHENOTYPES IN DIABETIC INDIVIDUALS WITH CARDIOVASCULAR DISEASES

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Several studies have demonstrated a marked increase in cardiovascular diseases in diabetic patients. However, all diabetic patients don't have the same risk. Haptoglobin is a serum protein that acts as an antioxidant by virtue of its ability to bind to hemoglobin and thereby to prevent the oxidative tissue damages that maybe mediated by free hemoglobin. Haptoglobin is a polymeric molecule that has three phenotypes (1-1,2-1 and 2-2). Biophysical and biochemical properties of the haptoglobin polymeric molecules are dramatically different. In human, two alleles (denoted 1 and 2) are responsible for these different phenotypes. It was recently demonstrated that an allelic polymorphism in the haptoglobin gene is predictive of the risk for numerous cardiovascular diabetic complications, but this effect is different in various populations. In this study for the first time we determined haptoglobin phenotypes in 61 male and 61 female Iranian diabetic patients with cardiovascular diseases and we showed that existence of the allele 2 is a predictor of the risk for cardiovascular complications in diabetic individuals.

p-643
MOLECULAR ANALYSIS of α-GLOBIN GENE IN CARRIER INDIVIDUALS REFERRED TO PASTEUR INSTITUTE OF IRAN

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There are four α-globin genes in normal individuals, arranged as pairs on the short arm of chromosome 16.The most

common cause of α-thalassemia is the deletion of one or more functional α-globin genes. The aim of this study was to determine α-globin gene deletions in Iranian individuals with low MCV&MCH and normal HbA2. The patients had been referred to us by different primary health care centers involved in national premarital screening for thalassemia. A total of 100 individuals were selected with low MCV&MCH and normal HbA2 who had no response to iron therapy. DNA was extracted from blood samples and used to perform Multiplex Gap-PCR for 4 common α-globin gene deletions. (-α3.7, -α4.2 ,- α20.5 ,--MED). The genotypes of these individuals were: 27 (-α3.7), 8 (--MED), 7 (- α4.2), and 5 (- α20.5), showing that microcytic hypochromic anemia in Iran is mostly caused by deletion (-α3.7). These results will be helpful in premarital genetic counseling of undetermined cases.

p-644
INTERACTION ANALYSIS OF THE SORTING SIGNAL DOMAIN OF PEX3P: FUNCTIONAL ROLE IN PEROXISOMAL SORTING OF PEX3P

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We have previously predicted that forty amino acid residue from the N-terminus of Rat PEX3 cDNA is adequate for peroxisomal sorting, which termed Pex3p-mPTS. Yeast two hybrid analyses failed to show any interaction of this domain with the other peroxins, proteins that are involved in peroxisome assembly and biogenesis, especially Pex19p that is known to act as a chaperone and interact with the other Peroxins-mPTS and directing them to the membranes of peroxisomes. In order to understand the molecular mechanism of peroxisomal directing of Pex3p, which seems to be independent of Pex19p, we have investigated intensive interaction analyses of this domain with the other peroxins using mammalian two-hybrid assay into the CHO K1 cells and CHO cells defective in peroxisome biogenesis. Here we show that the N-terminus of Pex3p can interact with the C-terminus of Pex16p which is one the main peroxins at early steps of peroxisome membrane formation. This interaction showed an increment when Pex19p was present. Similar data was obtained when Pex14p or Pex16p was used as the bait for interaction analysis with Pex3p-mPTS.

p-645
MOUSE PEROXISOMAL PROTEIN: CLONING AND ECTOPIC EXPRESSION OF A NOVEL MATRIX PEROXISOMAL PROTEIN

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Peroxisomes are single membrane bound organelle that ubiquitously distributed in eukaryotic cells and are involved in cellular detoxification reactions. Identification of peroxisomal matrix proteins and their import has deepened our understanding of the molecular biology of protein kinesis in peroxisomes. We previously indicated the importance of peroxisomal function in the cell has been underscored by the clinical documentation of a series of inherited recessive human disorders as zellweger syndrome. In this study, we have cloned murine cDNA of peroxisomal protein by the following approach. Total RNA was extracted from the brain tissues of an adult mouse. At the second step, cDNA was synthesized with oligo DT-primer and superscript transcriptase. Two steps of RT-PCR were done with appropriate primers introducing BglII and SalI sites at the head and tail of PCR products. Amplified PCR products were inserted into the mammalian expression plasmid, pEFGP-C1, under regulation of CMV promoter, fusing downstream of EGFP cDNA. Then we transiently transfected pEGFP-C1 containing EGFP-PEP into the CHO and P19 cells. These cells were compared with the empty vector transfectants. Numerous intracellualr particles presumably peroxisomes were discernible upon transfection of those plasmids into the mammalian cells. However there is no further evidence regarding to the structural and domain analysis and function of this protein. We are going to analyze its function during development and differentiations of the stem cells.

p-646
A REPORT OF PRENATAL DIAGNOSIS ON 4 SET OF TWIN'S THALASSEMIC CARRIER STATE

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Beta thalassemia is the most common heredity disease and is characterized by reduced synthesis or absence of beta-globin chain hemogolobin. The ferequency of beta-thalassemia carrier in Iran is 4-5%. Severest form B-thalassemia called thalassemia major that is transfusion dependent. Among all cases, which were referred to our lab, four mothers had pregnancy of twins. CV samples were obtained by transabdominal processing at 10-12 weeks of gestation. Mother parts were eliminated under the dissection microscope. DNA was extracted from CVS using standard technique. After characterizing parental mutions by ARMS and performing linkage analysis by polymorphic markers (RFLP), that results showed: For tow family, the twins were one normal, one minor.for the other family, both of twins were minor,and for the last family,one of twins was major and the other was minor.In the case if one found the same mution and RFLP in fetus and her/his mother a variable number of tandem repeat (VNTR) markers (PKU,APOB…..) were used to aviod mother contamination.That is very important point for surjon to know exact major twin, by the radiologist.

p-647
DETECTION OF β -GLOBIN GENE MUTATIONS IN PATIENTS WITH β-THALASSEMIA MAJOR IN KURDISTAN & KURDS POPULATION OF WEST AZERBAIJAN PROVINCES OF IRAN

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β-Thalassemia is a common autosomal-recessive disorder, caused by more than 200 different mutations of the β-globin gene, which results in either the absence of synthesis of the β-globin chains (β°- thalassemia) or a reduction in their output (β+- thalassemia). Different ethnic groups and tribes live in Iran and ethnic/genetic heterogeneity has resulted in a high number of different mutations that account for β-thalassemia. Comparison between different provinces shows that the mutation spectrum differs substantially in types and frequencies. Fifty-five Kurdish unrelated patients with known β-thalassemia major and intermedia, registered with the thalassemia clinics in Kurdistan and west Azerbaijan provinces, were included in this study. Mutations were studied in 110 chromosomes, by Polymerase Chain Reaction-Amplification Refractory Mutation System (PCR-ARMS), Single Strand Conformation Polymorphism (SSCP) and direct Sequencing methods. We found eleven β- thalassemia mutations in these regions of Iran. The results showed that IVS-II-1 (G>A) mutation was the most frequent, comprising 31% of all mutations. Other mutations were IVSI-1(G>A), FSC8/9 (+G), FSC8 (-AA), IVSI-110 (G>A), FSC36/37 (-T), IVSI-5 (G>C), IVSI-128 (T>G), FSC44 (-C), FSC 5(-CT) and +22UTR (C>T). Overall these mutations comprises 80% of β-thalassemia mutations and 20% of the mutations still await exploration. Our data show that the pattern of mutations in these regions is different from other Iranian Provinces. The results of our study will be useful to identify the β-thalassemia mutations, essential for genetic counseling and for organizing a prenatal diagnostic service in the Iranian Kurdish population.

p-648
MUTATION ANALYSIS OF COAGULATION FACTOR IX GENE IN ESFAHANIAN HEMOPHILIA B PATIENTS BY SSCP AND SEQUENCING

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Background and Objectives: Hemophilia B is an inherited recessive X-linked bleeding disorder caused by deficiency or defect of procoagulant factor IX (FIX). The factor IX gene spans 35 kb of DNA and comprises of 8 exons. Mutations in the factor IX gene may result in deficient or defective coagulation factor IX causing the bleeding tendency known as hemophilia B. The aim of this study was to identify the causative mutations and genotype-phenotype correlation for mutations in some known patients with hemophilia B in Isfahan province. Materials and Methods: After informed consent was obtained, genomic DNAs of 24 hemophilia B patients referred to Omid hospital were extracted according to standard protocols. PCR amplification and single strand conformation polymorphism (SSCP) on nondenaturing polyacrylamid gel were performed separately on each sample for eight exons and exon-intron boundaries and promoter. The results of SSCP were compared to normal control and sequencing was performed for those with different migration patterns. Results: The sequencing results showed 70.8% missense mutation, 16.7% deletion, 8.3% nonsense mutation, and 4.2% insertion. Many of the mutations had occurred in exon 8; it came out to be similar to haemophilia B mutation database. Malmo polymorphism (Ala 148 Thr) was found in one family. We found four novel mutations not previously reported. Conclusions: This study confirms the marked heterogeneity of factor IX mutations in the population. The results could be used to develop a national database and offer genetic counseling to families.

p-649
CONSTRUCTION OF MOUSE PEP CDNA CHIMERA WITH EGFP UNDER REGULATION OF CMV PROMOTER

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Peroxisome is ubiquitous organelle in all eukaryotic cells from yeast to human. The identification of peroxisomal proteins and mechanisms of their sorting, in the past decade has deepened our understanding of the biology of this tiny organelle and its involvement in human disorders. One of the previously recognized proteins is peroxisomal protein (Pep), which is expressed during myogenesis and brain differentiation. Amino acid alignment analysis revealed two hydrophobic domains. The first comprises twenty amino acid residues between 12-31 residues and the second one, is located at 152-169 residues. There is a tripeptide (SKI) at carboxy terminus responsible for sorting of this protein to the matrix of peroxisome. There is a fibronectin type III domain between residues 31-114 in pep. In order to see the importance of above sorting signal, we performed a site-directed mutagenesis to delete SKI tripeptide. Amplified Pep cDNAs either containing SKI or deleted ones were constructed downstream of EGFP cDNA under regulation of CMV promoter in pEGFP-C1 vector and were send for sequence. Transfection of plasmids containing chimera of EGFP-PEP cDNAs in to the CHO-K1 and P19 cells, showed several punctuate structures presumably peroxisomes while, SKI deletion showed a cytosolic mislocalization of EGFP pattern. Taken together, these data strongly suggest that SKI, which is located at the C- terminus of protein, is required for sorting of this protein.

p-650
NO INCIDENCE OF ALLELIC MUTATION IN BOOROLA (FECB) AND INVERDEL (FECXI) GENES IN LURI-BAKHTIARI SHEEP BREED

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High ovulation rate and litter size are the most important effective factors in reproduction system that increases economical efficiency in sheep breeding industry. This study was carried out for detection of mutation in FexB and FecXI genes in Luri-Bakhtiari sheep breed. It has been reported that these major genes can increase ovulation and also twining rate in different sheep breed. Blood samples were collected from

165 individual for identification of polymorphisms at these loci. DNA was extracted using modified salting out method and for amplification of desired fragments at these loci, two pairs of specific primers were applied for polymerase chain reaction (PCR). Each of specified primer pairs amplified a fragment with 190 and 154 bp at FexB and FecXI loci, respectively. For genotyping of samples, the PCR products after digestion with endonuclease AvaII for FecB and XbaI for FecXI were electrophoresed on 3% agarose gel. In the case of mutation two fragments of 30, 160 and 30, 124 bp could be detected in FexB and FecXI loci, respectively. Since all samples showed monomorph (+/+) genotypes the present study did not confirm the incidence of mutant allele (-) allele. Regarding the phonotypic record in this breed, the obtained results indicates that the genetic factor responsible for twining or multiple lambing rate is not related to reported mutated alleles at Boorola or Inverdel major genes and we should search for other genes in this breed.

p-651
IDENTIFICATION OF ALLELIC POLYMORPHISM IN OOCYTE-DRIVED GROWTH FACTOR (GDF9 AND BMP15) GENES IN LURI-BAKHTIARI SHEEP BREED

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The study of inheritance pattern of reproduction rate in sheep showed different breeds that have single nucleotide mutation in oocyte-derived growth factor GDF9, BMP15, and BMP15 proteins' receptor (BMPR-1B) genes. This study was carried out for detection of polymorphism in GDF9 and BMP15 genes in Luri-Bakhtiari sheep breed. Blood samples were collected from 165 individual of Luri-Bakhtiari sheep breed and transferred to laboratory using cooling chain and kept at -20°C till DNA extraction. DNA was extracted using modified salting out method and the quality and quantity of extracted DNA was measured by spectrophotometric and electrophoresis methods. Using specific primers in PCR reactions two fragments with the size of 141 and 153 bp were amplified for detection of FecXG and FecXB mutation from exon 2 of BMP15 gene. A fragment with of 462 bp was also amplified for detection of mutation from exon 1 of GDF9 gene. The PCR products were digested with specific restriction enzymes HinfI for BMP15 and HhaI for GDF9 site. The SSCP method was used for detection of FecXB mutation. The results from digested PCR products did not show mutation of FecXG. But the SSCP analysis indicated mutated FecXB allele in12% of genotyped samples. Almost all of the labeled ewes (62%) with FecXB allele in genotyped samples had twin or multiple lambing. HhaI restriction digestion of 462 bp fragment from exon 1 of GDF9 showed a 2% frequency of mutant genotype in this population. The preliminary results of the present study indicate the presence of mutant major genes related to oocyte-drived growth factors in Luri-Bakhtiari sheep breed. To confirm this hypothesis an experiment with a larger sample size and with phenotypic records should be conducted. Key words: Sheep, Luri-Bakhtiari, PCR, FecXB, FecXG, and GDF9.

p-652
EVALUATION OF P-GLYCOPROTEIN DRUG TRANSPORTER POLYMORPHISM AND ITS ASSOCIATION WITH COLORECTAL CANCER

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The human multidrug resistance (MDR1) gene product P-glycoprotein (P-gp) is highly expressed in intestinal epithelial cells. MDR1 constitutes a barrier against xenobiotics, bacterial toxins, drugs and other biologically active compounds, and possibly carcinogenes. In this study, we investigated the association of MDR1gene polymorphism and the occurrence of colorectal cancer. Methods: In this case-control study 118 unrelated colorectal cancer patients and 137 sex-and-age matched healthy controls were studied. The C3435T MDR1gene polymorphism was identified using the polymerase chain reaction-restriction fragment length polymorphism method. Results: Compared with controls significantly increased frequencies of the 3435T allele and the 3435TT were observed in patients with colorectal cancer (3435T: P=0.03; OR, 1.43; 95% CI, 1.02-2.8; 3435TT: P=0.003; OR, 2.2; 95% CI, 1.3-3.74). In contrast, frequency of TT genotype was significantly higher in controls compared to colorectal cancer (TC: P=0.006; OR, 0.49; 95% CI, 0.30-0.82). Conclusions: This study suggests that C3435T MDR1 polymophism has an association with colorectal cancer. The results support the notion that P-glycoprotein plays a major role in the defense against intestinal bacteria or toxins. Likely the presence of allele C results in decreased susceptibility to colorectal cancer.

p-653
DETECTION OF A NOVEL MUTATION IN TWO RELATED IRANIAN HEMOPHILIA B PATIENTS BY SSCP AND SEQUENCING

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Hemophilia B, Christmas disease is an X-linked bleeding disorder caused by the functional deficiency of blood coagulation factor IX. The disease is due to heterogeneous mutations in the factor IX gene (F9), located at Xq27.1. The gene spans about 34 kilobases (kb) of genomic DNA. After obtaining informed consent, genomic DNA was extracted from peripheral blood of two related severe hemophilia B patients (referred from Esfahan Hemophilia Center) by standard methods. PCR amplification and single strand conformation polymorphism (SSCP) technique were performed for scanning of the all functional-important regions of the F9 gene. An abnormal SSCP profile was identified in exon 6 of the F9 gene for the patients. Then, direct sequencing was done according to Sanger method. The nucleotide

substitution (T20513C) was detected in the patients. This change represented F178S (Phenylalanin 178 Serine), which has not been reported in haemophilia B mutation database, previously. We also designed a HinfI restriction fragment length polymorphism (RFLP) test that was performed for confirmation of mutation in patients and testing of their carrier mothers. The results showed that the mother is a carrier (as it is expected). The position of F178S mutation is in the activation domain of the protein, within which factor XIa, and VIIa plus tissue factor cleave proteins in 2 positions and convert factor IX to active form (FIXa).

p-654
THE SEARCH FOR FEMALE-SPECIFIC MORPHOLOGICAL EVENTS DURING OVARY DEVELOPMENT

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Before sex determination a bipotential gonad is formed with the capacity to differentiate into either testes or ovaries depending on the presence or absence of the Sry gene. Sry gene is active for a very brief period in somatic cells of the genital ridge in mouse that initiates a downstream events leading to testis formation. The molecular mechanisms regarding the activation of Sry and its target genes has been the major interest of scientific research for several years, which lead to the identification of numerous related genes, such as Sox9 and WT1. Furthermore, studies on SF1 expression in male somatic cells showed difference between developing Sertoli and Leydig cells. Morphological organization of various cell types within the developing ovary was shown to be less obvious. Therefore this study concentrates on obtaining a better understanding of the morphological events that occur during ovarian development. Histological and immunohistochemistry analyses in developing ovaries from early foetal to newborn stages were compared to male gonad littermates. These analyses indicated a number of ovary-specific changes of the gonad morphology that happens very early during ovary development. Moreover, a female-specific development of vasculature shortly after Sry expression reaches peak levels in gonads of male littermates that suggests an activation of female sex determining genetic pathways.

p-655
STUDY OF SOME MICROSATELLITE LOCI IN MYBOD QUAIL POPULATION

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To determine the genetic variation in four quail strain [Canadian Pharach (CP), Spanish Pharach (SP), English White (EW) and Manchurian Golden (MG)] from Meybod research station, we used seven high polymorphic microsatellite markers (GUJ0023, GUJ0083, GUJ0055, GUJ0099, GUJ0041, GUJ0059 and GUJ0052). DNA was extracted from whole blood samples collected from 150 quails. The PCR reactions were successfully done with all primers. All locus-strain combinations deviated from Hardy-Weinberg equilibrium except GUJ0023 in Canadian Pharach and Spanish Pharach strain, GUJ0099 in Manchurian Golden strain and GUJ0052 in Canadian Pharach and Manchurian Golden strains ($P<0.05$). The effective number of alleles per locus varied from 2.8064 (GUJ0099) to 4.4662 (GUJ0023). The highest and the lowest PIC values belonged to GUJ0023 in Spanish Pharach (0.6964) and GUJ0083 in English White strains (0.4415) respectively. The expected heterozygosity varied between 0.7438 and 0.5450. The lowest and highest DS, Rogers, Wright and co-ancestry genetic distances were obtained between (CP) with (SP) and (EW), respectively. The phylogenies based on DA, DS, Rogers, Wright and co-ancestry distances by unweighted pair-group method using an arithmetic average (UPGMA) showed CP, SP and MG are together at one cluster and EW at another. We have described informative quail microsatellite markers that would form a useful resource of DNA markers as part of our initiative to develop a genetic map for quail. Furthermore, the microsatellite markers reported would serve as a useful resource for genetic mapping in quail and comparative mapping in Phasianidae.

p-656
STUDY OF ITPA GENE ACTIVITY IN CML CANCEROUS CELL LINE (K-562)

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Inosine triphosphatase (ITPA) is a ubiquitous key regulator of some non-canonical cellular purines. It breaks down inosine and xanthine triphosphate nucleotides to their corresponding monophosphate forms, generated by deamination of purine bases. Its enzymatic action prevents accumulation of ITP and reduces the risk of incorporation of potentially mutagenic inosine nucleotides into nucleic acids. ITPA orthologs are found in organisms from all kingdoms. It has been shown that ITPA deficiency leads to an increased level of ITP, which may be harmful under circumstances such as cellular stress. Although a complex method, HPLC has been frequently used for ITPase assay. To develop a simpler and quicker one, we devised a luminometry-based method for evaluating the activity of ITPase. At the first phase of the research, expression of recombinant protein of hITPase was induced and its activity was evaluated. Moreover, it has been shown that CML (K-562) cell line has genetic instability and since disorders in ITPA gene function can lead to mutation increase in genetic materials of cells so the expression of this gene has been considered as a cause of genetic instability in this study. In the second phase of the research the expression of ITPA in K-562 cell line was examined. For this purpose, the cDNA obtained from K-562 cell line was amplified by the specific primers of ITPA gene. Sequence analysis showed two forms of cDNA for ITPA gene in K-562 cell line. One was similar to

variant 1 ITPA gene and the other contained a deletion in a part of exon one & two. This deletion seems to be a result of an unknown splicing or a missplicing in this new form. We conclude the function of ITPA gene dose not follow a normal procedure. Therefore, the activity of protein can be considered as a probable factor for increasing mutation and genetic instability in this cell line.

p-657
HB D-PUNJAB [β 121 (GH4) GLU→ GLN] / β0-THALASSEMIA (IVSII.1.G→ A) IN TWO CASES FROM A FAMILY: FIRST REPORT FROM IRAN

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Molecular and hematological characteristic of two boys (3 and 10 years old) with Hb D-Punjab/ β0- thalassemia (IVSII.1.G→ A) from a family living in Western Iran in which father was Hb D-Punjab carrier and mother was a minor β-thalassemia is described. Haplotype analysis indicated that the 3 βD-Punjab chromosomes were linked to haplotype I. While the 3 β-thalassemia chromosomes were associated with atypical haplotype [- + + + + + -]. The Xmn I polymorphic site 5 to Gγ gene was absent on the βD-Punjab chromosomes. However, the Xmn I site was present on the β-thalassemia chromosomes. The hematologic values of these two complex heterozygous patients were a high levels of Hb D-Punjab (76.7, 78.1%, respectively), significant elevation of Hb F (18.1 and 16.6%, respectively), significant reduction in MCV (56, 61.0 fL, respectively) and MCH (19.5, 20.1 pg, respectively), and presenting moderate anemia.

p-658
NEW GENETIC ANALYSIS FOR MENTAL RETARDATION IN IRAN

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Mental retardation is one of the main reasons for referral for pediatrics (children), to neurological and clinical genetic practice. On the basis of the observation that mental retardation is significantly more common in males than in females, X-linked gene defects have long been considered to be important causes of mental retardation. X-linked mental retardation (XLMR) has a prevalence of 2.6 cases per 1,000 in the general population, accounting for over 10% of all cases of mental retardation. It is estimated that 2/3 of X-linked mental retardation is non-syndromic (mental retardation without other distinguishing features). Clinical observations and linkage studies in families revealed that X-linked mental retardation (XLMR) is a highly heterogeneous condition. The most common form of XLMR — the Fragile X (Fra(X)) mental-retardation syndrome is associated with a cytogenetic marker in the distal region of the long arm of the X chromosome, which was shown to coincide with the map position of the underlying gene defect, and eventually this led to the cloning of the FMR1 gene. The fragile X syndrome is the most frequent cause of inherited mental retardation. It is caused by a dynamic mutation: the progressive expansion of polymorphic (CGG)n trinucleotide repeats located in the promoter region of the FMRI gene at Xq27.3 .The fragile X syndrome was the first triplet repeat disorder identified and served as a prototype for several diseases caused by triplet repeat expansions in the human genome. FXMR primarily affects males but approximately one-third of the carrier females are also found to be affected, though the severity of mental retardation in females is less than in the males. There are some fragile X patients who have normal repeat but small deletion or point mutation is in related gene. We studied FMR1 and MECP2 gene with linkage analyses with three markers for eacth gene in five families. Markers for FMR1 gene are FRAXAC1, FRAXAC2 and DXS8091. Maximum LOD score is 5.83 for FRAXAC1. Markers for MECP2 gene are DXS1108, DXS52 and DXS7096. Maximum LOD score is 5.43 for DXS1108. This study shows that the frequency of mutations in MECP2 in the mentally retarded population screened for the fragile X syndrome is comparable to the frequency of the CGG expansions in FMR1. Therefore, implementation of systematic screening of MECP2 in MR patients should result in significant progress in the field of molecular diagnosis and genetic counseling of mental retardation. KeyWords: M.R, IRAN, MECP2, FMR1 gene.

p-659
E-CADHERIN/ESTROGEN RECEPTOR GENE PROMOTER HYPERMETHYLATION AMONG IRANIAN SPORADIC BREAST CANCER

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Cancer development can be traced to epigenic changes. The main epigenetic modification in human is methylation of cytosine nucleotides. A cell's DNA methylation balance is dramatically altered in cancer. Tumor suppressor genes are unmethylated in normal tissue and often become hypermethylated during tumor formation, leading to gene silencing. Aberrant methylation of E-cadherin (E-cad) and estrogen receptor (ESR1) 5´ CpG islands was evident in sporadic breast tumors. In an effort to determine the CpG islands hypermethylation of these genes in Iranian population, we studied on 48 primary breast cancer samples from the department of pathology at Shiraz universityof Medical Sciences. Genomic DNA was extracted from tumors and treated with sodium bisulfate to use as template in methyl-specific PCR for E-cad and ESR1 genes. A total of 48 primary breast tumor samples were identified as 43 infiltrating ductal carcinoma (IDC), 3 infiltrating lobular carcinoma (ILC) and 1 in situ ductal carcinoma. The majority of the breast cancer (58%) showed aberrant methylation in at least one of the two tested loci. Methylation of the E-cad gene was observed in 41% (18 of 43) of IDC samples, whereas ESR1 methylation was observed in 26% (11 of 43). Coincidence methylation was present in only 12% of these ICD lesions. All of 3 ILC samples and an in situ ductal carcinoma sample showed only

methylation of the E-cad 5′ CpG islands. In comparison to clinical data we observed that methylation of E-cad CpG island increases with cancer progression. CpG island hypermethylation of E-cad may have three possible translation uses: as a marker for cancer cells, as a predictor of tumor behavior and as a predictor of tumor response to therapeutic interventions.

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DEVELOPING MOLECULAR GENETIC PROFILING OF 16 MICROSATELLITE LOCI FROM DENTAL DNA FOR THE FIRST TIME IN IRAN

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Repetitive sequences of DNA throughout the human genome occur in about 10% of total genome and also lead in to generation of certain polymorphisms. These sequences are used as an important genetic maker in investigation of gene mapping, diagnosis of diseases, matching suspects with controls, determination of family relation, historical investigations, unknown personal identity, and etc. The STR1 sequences include those short sequences that consist of 2-6 bp repetitive units and also they can be identified and amplified by PCR. STRs are widely used in research and forensic medicine laboratories all over the world. DNA extraction from hard tissues like tooth has always been so complicated that many researchers did not apply it. Teeth and bones have been shown to be valuable sources of DNA samples. Dental enamel, the hardest substance in the human body, protects the DNA rich pulp and dentin and therefore ensures a good quality of isolated DNA. In this work for the first time in Iran, genomic DNA was extracted from tooth hard tissue samples and 16 STRs were profiled. High constancy of bone tissue enables its application for identification of dead bodies, which have been past a long time. It should be mentioned that the remains of dead bodies are the product of natural disasters like flood and earthquake and unnatural calamities like war.

O-661

MAKING A CONSTRUCT CONTAINING A MINI LCR & β- GLOBIN GENE EXPRESSION CASSETTE AIMING FOR GENE THERAPY OF BETA-THALASSEMIA

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β-Thalassemia is an autosomal recessive disease caused by absence or reduction of β-globin chain synthesis. The disease is the most common monogenic disorder in Iran. The β-globin gene is located at 11p in a cluster controlled by locus control region (LCR) about 50 kb upstream of the β-globin gene. The LCR composed of five DNase I hyper sensitive sites (HS) in Erythroid cells and is required for high level expression of genes in globin gene cluster at all developmental stages. More than 180 mutations in β-globin gene and some deletions that remove a part or all of the LCR, detected in β- thalassemia patients. In this study, we made a construct containing essential regions of LCR (HS2- HS3- HS4) and β-globin gene with 5' and 3'- untranslated regions (UTR) of the gene (accession no. NG_000007 from 70283-72389). Among of hyper sensitive sites, HS3 is more important in high expression of β-globin gene. After amplification of these segments from the genomic DNA of a healthy person by PCR, cloning was done in the pBGGT vector, a derivative of PUC19 plasmid; DNA sequencing and restriction analysis with different enzymes were used for cloning verification. The final construct could be used for making a construct that is used for gene therapy of β-thalassemia. In addition this construct could be used of assessment of nucleotide changes in 5' and 3' –UTR of β-globin gene, and also be exploited for testing the cryptic splice sites in exons and introns of β-globin gene.

p-662

MOLECULAR CHARACTERIZATION OF A HSP60 GENE IN DERMATOPHYTE PATHOGEN MICROSPOROM CANIS

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The stress-inducible Heat Shock Proteins (i.e. HSP60) constitute one of the highly conserved protein and gene families. As one of the molecular chaperone proteins, they play essential roles in protein metabolism and protein translocation under both stress and non-stress conditions. In the present study we tryed to characterize the 60 kDa Heat Shock Protein (HSP60) gene in dermatophyte pathogen Microsporom Canis (M. canis). This dermatophyte is one of the most important cause agents of dermatophytosis in human and animals. Some properties of M. canis have been investigated in molecular level; however, no information is available regarding the HSP60 in this dermatophyte. M. canis was obtained from patients with dermatophytosis and cultured in appropiate conditions. High molecular weight DNA was isolated from obtained mycelial mass by standard methods. Pairs of 21 nt primers were designed from highly conserved regions of the HSP60 genes in other eukaryotic cells. The primers were applied for PCR reactions using isolated genomic DNA template of M. canis. Predicted molecules have been amplified and were submitted for sequencing. Almost 1550 bp fragment of the DNA encoding a 497 amino acids protein has been sequenced. Nucleotide sequence comparison in gene data banks (NCBI, NIH) for the DNA and its deduced amino acid sequence revealed significant homology with members of the eukaryotic HSP60 family. Nucleotide and amino acid sequences of McHSP60 have been submitted to the National Centre for Biotechnology Information GenBank and are available for public access under the accession Number: DQ981834.

p-663
DETERMINATION OF MOLECULAR STRUCTURE OF THE 60 KD HEAT SHOCK PROTEIN GENE IN DERMATOPHYTE PATHOGEN TRICHOPHYTON RUBRUM

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Cell exposure to some stress conditions such as heat shock, oxidant injury, heavy metals, and ultraviolet radiation results in activation of heat-shock genes and synthesis of heat shock proteins. In the present study, we tried to characterize the 60 kDa Heat Shock Protein (HSP60) gene in dermatophyte pathogen Trichophyton rubrum (T. rubrum). This dermatophyte is the most common cause agents of dermatophytosis in human skin and nail tissue. Some properties of T. rubrum have been investigated in molecular level; however, no information is available regarding the HSP60 in this dermatophyte. T. rubrum was obtained from patients with dermatophytosis and cultured in appropiate conditions. High molecular weight DNA was isolated from obtained mycelial mass by standard methods. Pairs of 21 nt primers were designed from highly conserved regions of the HSP60 genes in other eukaryotic cells. Mentioned primers were utilized in PCR by using isolated genomic DNA template of T. rubrum. Predicted molecules have been amplified and were submitted for sequencing. The 864 bp DNA fragment encoding a 288 amino acids protein has been sequenced. Nucleotide sequence comparison in gene data banks (NCBI, NIH) for the DNA and its deduced amino acid sequence revealed significant homology with members of the eukaryotic HSP60 family. Nucleotide and amino acid sequences of TrHSP60 have been submitted to the National Centre for Biotechnology Information GenBank and are available for public access under the accession Number: DQ981835.

p-664
DETECTING AND EXAMINING THE POTATO Y VIRUS IN POTATO CULTIVATION FARMS OF KERMANSHAH

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The Potato Y virus is one of the potyviruse species with flexible particles and a helix structure. This virus is one of the most harmful potato viruses, which cause the Rogus disease. The symptoms of this disease are brown lines especially in leaves and in severe cases of the disease, falling of leaves occurs. In this study the RT-PCR method was used to detect the Y virus in potato tubers and leaves. Samples suspicious of viral infection were gathered from farms in Kermanshah and placed in plastic bags in -80˚ C temperature in order to preserve the viral RNA till extraction. The extraction and purification of RNA was carried out using Tri-Reagent kit. One of the virus genes is the P1 proteas gene, which codes a proteinase enzyme. This enzyme plays a role in breaking the initial polyprotein. For amplification of this gene three primers with the following sequences were designed: primer1: 5' GAGTTCCTGTGAAACCTT 3', primer 2: 5' CTTCATCAAACAACTCTTT 3', and primer 3: 5' GTTCTGACTACGGGTCTA 3'. Using primer 1 and reverse transcriptase enzyme, cDNA was synthesized and then by using primers 1, 2, and 3, PCR was performed. The PCR products were examined by agarose gel electrophoresis (1%). Two DNA fragments of 400 and 800 bp that were identical to the genomic DNA sequence were produced. Thus, the proposed technique is a convenient method for quick and accurate detection of viruses. The application of this method for detecting Potato Y virus in potato farms is recommended.

p-665
THE GENETIC RELATIONSHIP AMONG ELEVEN IRANIAN ETHNIC GROUPS: AN ANTHROPOLOGICAL VIEW BASED ON HLA CLASS II GENE POLYMORPHISM

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Besides being considered by immunologists for its pivotal role in the immune response, highly polymorphic HLA genes are regarded as useful markers for determination of genetic relationship and interaction among different populations by anthropologists. Knowledge of the HLA allele distributions in various populations is also critical for establishing bone marrow donor registry, studying of HLA associated disease and designing of peptide vaccines against infections, tumors, or autoimmune diseases. In this study, HLA class II profiles were determined in 816 DNA samples from eleven ethnic groups of Persia and the genetic relationship of Iranians compared to Asians and Europeans by molecular methods. DRB1*1103=04, DQA1*0501, and DQB1*0301 were the most frequent alleles and DRB1*1103=04-DQA1*0501-DQB1*0301 was the most common haplotype in Persia. Six samples typed as DRB1*1605 by direct sequencing of exon 2 and all of them were heterozygote for DRB1 locus and belonged to DRB1*1605-DQA1*0101/2-DQB1*0502 haplotype. The results of neighbor-joining and corresponding analysis revealed a close genetic relationship among Iranian subpopulations that were well separated from other Asians and European populations. The results of AMOVA also revealed that the main variation component (96.9%) was contributed by the within-population level and genetic differentiation (FST) was 0.03 when all Iranian subpopulations considered as one group.

p-666
FINDING PHYLOGENETIC RELATIONSHIP OF LIZA ABU AND LIZASUBVIRIDIS FROM NORTH OF PRSIAN GULF BASED ON MITOCHONDRIAL SEQUENCE DATA

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In this study genetic variation of two species of mullet (Liza abu & Liza subviridis) investigated by mtDNA sequencing method. For this purpose 16 samples were collected from Persian Gulf in Khuzestoan and Bushehr provinces. Their genomic DNAs were extracted from muscle tissue by phenol – chloroform method. For PCR amplification of D-loop region, the degenerate PCR primers were used to amplify the mitochondrial region (D- loop). Amplified PCR fragments were analyzed by agarose gel (1%) electrophoresis. PCR products were extracted from agarose gel, purified and sequenced. Then phylogenetic tree was drawn by inter and intraspecies sequence comparison. Phylogenetic tree revealed that there is far genetic distance between these two species. Therefore they are classified in seprate branches.

p-667
DETECTION OF MEDITERRANEAN MUTATION IN G6PD DEFICIENT INDIVIDUALS IN KHUZESTAN PROVINCE

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Glucose-6 phosphate dehydrogenase (G6PD) deficiency is one of the most prevalent genetic and enzymatic disorders in human. G6PD is inherited in an X-linked recessive pattern and encodes a housekeeping enzyme, which is vital for cell survival. G6PD catalyzes the first step of pentose phosphate pathway. Main clinical manifestations of this disorder are acute hemolytic anemia and jaundice induced by infection or ingestion of fava bean and certain drugs. According to previous investigations, Mediterranean mutation (563 C T) of G6PD gene is the most prevalent mutation in some provinces of Iran and neighboring countries. We chose this mutation to determine its incidence rate in Khuzestan province. In this study we have analyzed the G6PD gene in 42 blood samples (32 males, and10 females) from individuals with a history of fauvism from different ethnic groups of Khuzestan population. This mutation develops a new Mbo II restriction endonuclease recognition site in exon #6, so to identify this mutation we used PCR-RFLP method and Mbo II enzyme. We showed that 30 samples had the Mediterranean mutation. Therefore as we estimated, its frequency approximately is 71.4% and we suppose that the Mediterranean mutation has the most incidences in this population.

p-668
THE INVOLVEMENT OF AGGRECAN POLYMORPHISM IN DEGENERATION OF HUMAN INTERVERTEBRAL DISC

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The functions of the intervertebral disc and articular cartilage are intimately related to their aggrecan content. This function is related to the structure that posses variable number of tandem repeat (VNTR) polymorphism in exon 12 encoding the CS1 domain. Alleles are aggrecan, and in particular to the large number of chondroitin sulphate chains present on its core protein. The human aggrecan gene is unique amongst species that identified with CS1 repeat ranging from 13 to33. The most common alleles possess 26, 27 or 28 repeats. Individuals possessing the shortest alleles will have the lowest number of CS chains and this could lead to a lower ability to withstand compression and an increased susceptibility to tissue degeneration. In this research we used PCR for determining CS1 polymorphism. Statistical analysis of 20 patients and 20 controls indicated that 26(%17.5) and 27(%27.5) the most alleles had the repeat between our study groups. MRI examination showed that 9 patients had disc degeneration with spondylolysis, 6 individuals had disc degeneration without spondylolysis and 5 individuals had sever disc degeneration with spondylolysis. We did not find the lower range of allele size in the disease groups. Thus, no correlation was found between aggrecan CS1 polymorphism and degeneration.

p-669
RELATIONSHIP BETWEEN ACE GENE POLYMORPHISM AND RAPIDITY OF PROGRESSION OF FOCAL SEGMENTAL GLUMERULOSCLEROSIS TO END STAGE RENEAL DISEASE IN IRANIAN CHILDREN

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Introduction: Idiopathic focal segmental glomerulosclerosis "FSGS" is one of the most common glomerular diseases leading to end stage renal disease (ESRD) in children. These patients show different rate of progression. Angiotensin converting enzyme (ACE) gene insertion/deletion (I/D) polymorphism has been studied as a predictor of progression of renal diseases including FSGS. However, the studies have controversial results. There is no study available from our country so far to find out any correlation of ACE gene polymorphism and rate of progression in FSGS children. Aim: We investigated its role in the rate of progression of FSGS. Method: Forty-one children aged 1 year to 18 years admitted at St AlZahra Hospital, Isfahan Iran with biopsy proven idiopathic FSGS were enrolled. They were divided into two groups according to the time of progression to renal death. Renal death was defined as glomerular filtration rate (GFR) less than 50 ml/min/1.73m ^{2} or decreasing GFR more than two times compare to the baseline. Reaching renal death in less or more than two years was assumed as rapid progressors (RP) or slow progressors (SP), respectively. The intron 16 of ACE gene was amplified by PCR technique. Statistical significance was regarded as $P < 0.05$. Results: Twenty-eight patients were male and 13 were female. In 15 RP patients the genotype

distribution was DD-26.6%, II-6.6%, ID-66.6%. In 26 SP patients the genotype was similar (DD-38.4%, II-7.6%, ID-53.8%, P> 0.05). Conclusion: We could not show any significant difference between gene polymorphism and rapidity of progression of FSGS in Iranian children.

p-670
THE INVESTIGATION OF GENETIC VARIATION IN RAENI CASHMERE GOATS USING EIGHT MICROSATELLITE MARKERS

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Raeini Cashmere goat is one of the most important breeds of Cashmere goat in Iran that is spread in Kerman and eastern area of Fars. The purpose of this study was investigation of intrapopulation variation of Raeini Cashmere goat. The study was conducted on overall 120 goats. The eight microsatellite markers were amplified by polymerase chain reaction (PCR), followed by 8% denatured polyacrylamid gel electrophoresis. An average of heterozygosity was estimated as 0.80. Five of the studied loci (LSCV11, OraFCB20, ILSTS059, IL2RA, ILSTS034) were at Hardy-Weinberg disequilibrium (P<0.005). The study of Shannon index and PIC (Polymorphic Information Content) also indicated the least and the most diversity for IL2RA and OraFCB20 loci, respectively. In general, it can be concluded that Raeini Cashmere goat has approximately high genetic diversity. Therefore for designing a breeding project needs attention to conserving the genetic diversity, so the genetic resources will be conserved as a world's national investments.

p-671
USE OF GYRB GENE SEQUENCES TO DETERMINE PHYLOGENETIC RELATIONSHIP

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Introduction: The genus salmonella forms a single DNA homology group with seven subgroups. The division of salmonella in to these groups has been confirmed by sequencing analysis of 16S rRNA gene. But this gene not only differentiates closely related species but also could be used to compare taxa level to kingdom. The DNA gyrase is more useful for classifying or detecting closely related organisms. Aim: we performed phylogenetic analysis using gyrB sequences to investigate the genetic relevance among isolates of salmonella enterica species. Material and method: Thirty six salmonella sp. Isolated from eight hospitals of Tehran were subjected to biochemical and serological tests. After DNA extraction by phenol-chloroform method, partial coding region of gyrB gene was amplified using primers UP1Fs (5′-GAAGTCATCATGACCGTTCTGCA-3′) and UP2Rs (5′-AGCAGGGTACGGATGTGCGAGCC-3′). The results of direct sequencing were analyzed by DNASIS software. All data was aligned by CLUSTAL X (1.8) and translated to amino acid by bioedit software. Result: According to the data of DNA sequencing, genetic distances were calculated by MEGA3 software. Our result showed that strain belonging to the same species of S.enterica have similarity of 98-100% and 99.4-100% for amino acid and nucleotide sequences, respectively. Homologies between the strains of two subspecies were quite low (96-97.3%). Conclusion: Analysis of gyrB sequence may be useful for sub-classification of organisms because the rate of evolution of gyrB is higher than 16S rRNA. To analyze the phylogeny of closely related bacteria such as salmonella, nucleotide sequences may be superior to that of amino acid sequences, as higher number of substitution are found in the former sequences.

p-672
A MUTATION IN AN ARABIDOPSIS CYSTEINE SYNTHASE CAUSES ENHANCED LEAF SENESCENCE

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Leaf senescence is a highly regulated programmed process that involves ordered sequential changes in cellular physiology, biochemistry and gene expression. In an attempt to better understand the mechanism of leaf senescence, we identified and characterized the Arabidopsis early leaf senescence mutant old3 (for onset of leaf death 3). In old3 plants, a rapid drop in cotyledon chlorophyll content, followed by an increase in ion leakage, occurred between 12-14 days after germination, while similar changes were not observed in wild type plants. The old3 mutant was isolated from the Ler-0 ecotype and the old3 gene showed different phenotypes in several other Arabidopsis ecotypes. Further analysis revealed that there is a specific interaction between the old3 gene and a Ler-0 gene called ODD (for old3 determination). The ODD gene was found to be a co-dominant gene and is located on chromosome 3. It was discovered that the OLD3 gene encodes a cytosolic cysteine synthase and a gly162 to glu162 substitution caused the old3 phenotype. The mutation resulted in a loss of function protein as judged by the inability of the old3 protein to complement a cysteine synthase E.coli mutant and by in vitro activity measurement of the OLD3 and old3 proteins. Since cysteine is the key step for the formation of organic sulphur-containing compounds, we examined leaf senescence and sulphur-associated physiology. We showed that in addition to the old3 gene, the ODD gene is required to cause changes in sulphur metabolism and leaf senescence and the results will be presented.

p-673
CARDIAC DIFFERENTIATION OF P19CL6 STEM CELLS BY OXYTOCIN

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The P19 embryonal carcinoma stem cells have been reported to differentiate into beating Cardiomyocytes by oxytocin. It

has been suggested that DMSO acts via the oxytocin pathway because an oxytocin antagonist not only blocks oxytocin induced cardiac myocyte differentiation, but also DMSO-induced differentiation. In this study we demonstrate that oxytocin can't induce differentiation of confluent monolayer culture into cardiomyocytes. On the other hand with prior EB formation, GFP expressed cardiomyocytes were produced by oxytocin with high efficiency. P19cl6 cells were cultured as confluent monolayer and aggregated cells. Then oxytocin with 1×10^{-7} M concentration added to culturing media as an inducer agent .The cells treated with 1% DMSO were used as positive controls. Differentiated cells were evaluated by morphological, immunocytochemical and RT-PCR evaluations. In continuing of this research the pEGFP-C1 plasmid was transfected into P19cl6 cells by means of electroporation method and stably GFP expressed cells were differentiated into beating cardiomyocytes by oxytocin. The results indicated that in experimental group, aggregates from P19CL6 cells could be differentiated into cardiomyocytes whereas monolayer cells couldn't. In control group both aggregates and monolayer cells could be differentiated into cardiomyocytes by DMSO. On the other hand P19cl6 cells, which efficiently were transfected by GFP, could be differentiated into beating cardiomyocytes by oxytocin. The results of all evaluations were confirmed that differentiated cells were cardiomyocytes. Our results show that embryoid body formation is necessary for differentiation of P19cl6 cells into cardiomyocytes when the cells are treated by oxytocin. It can be concluded that probably differentiation pathways for oxytocin and DMSO aren't absolutely the same. These results justify the continued study of the role of aggregation and oxytocin in process of cardiac differentiation.

p-674

A SINGLE NUCLEOTIDE POLYMORPHISM -1131 T > C IN THE APOLIPOPROTEIN A5 GENE ALTERS TRIGLYCERIDE METABOLISM

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The recently identified apolipoprotein AV (encoded by the ApoA gene) plays an important role in triglyceride (TG) metabolism. The common -1131C>T single nucleotide polymorphism in ApoA5 gene promoter affects plasma TG levels in several populations, and the association of the -1131C allele with increased TG levels is ethnic-specific. Objective: To determine the frequency of ApoA5 -1131C>T genotypes in a sample of unrelated Iranian population and to test whether the -1131 C>T allelic variants could affect fasting TG levels. Methods: DNA was extracted from 70 Iranians and analyzed for the −1131T>C polymorphism. The −1131T>C genotype was determined by PCR and restriction analysis. Plasma TG level was measured in the fasting state. Results: Plasma triglycerides was significantly higher in individuals with genotypes containing the minor allele of the −1131T>C polymorphism compared to the homozygotes for the major allele. Conclusions: ApoA5 -1131C>T variants are associated with fasting TG levels in Iranians and therefore affect TG metabolism in this population.

O-675

MOLECULAR ANALYSIS OF REGULATION OF BACTERIOFERRITIN GENE EXPRESSION IN E. COLI USING LACZ REPORTER GENE

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Iron is an essential nutrient but posseses problems of toxicity and poor bioavailability for aerobic microorganisms. Escherichia coli counteracts these problems by employing at least two iron-storage proteins, FtnA (ferritin), which acts as a major iron store, and a haem-containing bacterioferritin (Bfr) whose role is not clear. The global-iron regulator protein, Fur (ferric uptake regulor) regulates the expression of many iron-response genes including the ftnA and bfr genes, which encode FtnA and Bfr respectively. Fe2+-Fur complex represses transcription by binding to a 19-bp sequence (iron box) normally located near the Pribnow box of cognate promoters. Fur can also act as a transcriptional activator switching on several genes including bfr and ftnA. The mechanism of this activation is not clear and seems to be indirect because there is no iron box. A 71-bp-intergenic region is located between the bfr and its upstream gene (bfd). Herein, we studied the intergenic region to find a regulatory sequence effecting the bfr expression. To enable such analyses, different DNA fragment were made from bfr gene by cutting som nucleotids each time from bfr-bfd intergenic region gene plus some modification of them so different constructs were made and have inserted in codding sequence of vector containing lac Z as reporter gene so we expressed them in wild typ strain (W3110). The fragments were then inserted in the coding sequence of a lacZ reporter gene and the resulting constructs were expressed in wild type strain (W3110) and the b-Galactosidase activities were measured by ELIZA reader. The results showed a sequence that affect regulation of bfr gene expression. We found a sequence at the begining of the intergenic region that its bfr expression is very low. Therefore, it has regulatory role for bfr expression with or without iron at culture.

p-676

PHYLOGENETIC ANALYSIS OF THE cDNA ENCODING LUCIFERIN REGENERATING ENZYME FROM IRANIAN FIREFLY LAMPYRIS TURKESTANICUS

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The Luciferin Regenerating Enzyme (LRE) is an enzyme for regenerating D-luciferin in the firefly lantern. In the firefly light organ, oxyluciferin, a product of light emitting reaction of firefly luciferase is converted to D-luciferin. Oxyluciferin has a strong inhibitory effect on luciferase in a manner competitive with luciferin because of their high structural similarities. Based on these, conversion of oxyluciferin by LRE leads to the rapid turnover of luciferase for the next light emission. The luminescence intensity is increased with LRE compared with its absence. In this study to obtain the middle part of the cDNA which encoding LRE form lampyris turkestanicus, pairs of primers has been designed for RT-PCR and we appled RACE-PCR to obtain a part of the sequence located at the 3′ end. After sequence identification of these two parts of the cDNA from Iranian firfly Lampyris turkestanicus a homology search in the Swiss-Prot protein sequence database revealed no matches. These results indicated that the two parts of the cDNA encoding a novel protein. Phylogenetic analysis showed LRE from lampyris turkestanicus is in the same cluster as AFP (Anterior fat body protein). The remaining part of the cDNA sequence encods LRE. The identification of this part using RACE-PCR and final phylogentic anlysis will be reported.

p-677
BACTERIAL FLAGELLA GLYCOSYLATION IN AEROMONAS SPECIES

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Most mesophilic Aeromonas species produce two distinct flagella systems. In liquid, aeromonads are motile through a single polar flagellum (Fla), but on a solid surface they express an entirely distinct lateral flagella (Laf) system that is used for swarming. The polar flagellum requires over 40 genes for its biogenesis and assembly whereas lateral flagella need around 35 genes. The flagellins (structural proteins of the filament) of each flagella system migrate at an aberrant size on SDS-PAGE and are thought to be post-translationally modified. In Aeromonas caviae Sch3N the genes required for this modification are located in the flm locus, rmlB, flmA, flmB, neuA, flmD, neuB, lsg and lst. The predicted function of the translated products shared homology to proteins involved in polysaccharide biosynthesis or protein glycosylation. The locus was flanked by two transposon-like elements and had a low G + C (42%) content for Aeromonas (normally 60%) and is believed to have been acquired by horizontal transfer. Non-polar insertion mutants were created in all eight genes and they all demonstrated phenotypes of non-motility, lack of LPS O-antigen and flagella with the exception of the mutants in lsg and lst that only lacked their LPS O-antigen. The results suggest a locus with dual function with the genes lst and lsg being involved in LPS assembly only, whereas flmA, flmB, neuA, flmD and neuB are involved in saccharide biosynthesis that is required for both the LPS and flagella glycosylation. In the Aeromonas hydrophila AH-3, the orthologous genes are required for flagella only. Mass spectrometry of A. caviae polar flagellin tryptic digests has demonstrated that aeromonad flagella are indeed glycosylated with 6 – 10 residues of pseudaminic acid (5,7-diacetatamido-3,5,7,9-tetradeoxy-L-glycero-L-manno-non-ulosonic acid) a nine-carbon sugar that is related to sialic acid (Neu5Ac). Mass spectrometry has also shown that this unusual sugar is also missing from the LPS of the A. caviae flm locus mutants. Pseudaminic acid is found on the flagella of Helicobacter pylori and Campylobacter jejuni. Bacterial flagellin proteins interact with toll-like receptor-5 (TLR-5) on host cells and stimulate an inflammatory response. This results in interleukin-8 (IL-8) release, a measure of cell stimulation. Therefore pseudaminic acid on aeromonad flagella may mask these structures from the immune system. Aeromonad flagellins with and without pseudaminic acid additions were engineered and tested on the human cell line Caco-2, which has the TLR-5 receptor. Both unglycosylated flagellins, (FlaA and FlaB), were confirmed to stimulate IL-8 production and up-regulation of TLR-5 expression.

p-678
PREPARATION OF MUTANT FORM OF FVII FOR GENE THERAPY OF HEMOPHILIA

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Hemophilia is one of the most common inherited disorders of blood coagulation which is caused by factor VIII (hemophilia A) or factor IX (hemophilia B) deficient activity. Treatment for hemophilia currently involves replacement therapy using blood-derived concentrates of FVII and FIX, but continuous injection of blood factors develops neutralizing antibodies (as referred to inhibitors) against infusion proteins. Moreover, these concentrates have been associated with viral infections and thromboembolic complications. More recently, recombinant activated human factor VII (rhFVII) used for the treatment of hemophilia A or B patients. Inhibitors are hemostatically effective because it induces thrombin generation, despite impaired FVII and FIX-dependent amplification of FX activation. However, its short half-life necessitates frequent infusions and results in high treatment cost. One potential solution to this problem may lie in the use of FVIIa gene transfer, which would achieve long-lasting therapeutic levels of expression from a single injection. We engineered a novel FVII gene containing a cleavage site for the intracellular protease, furin, by PCR mutagenesis, which was verified by restriction enzyme, and sequencing and cloned this construct into a cloning vector and transformed in top10 strain of Ecoli. This mutant form of FVII can be used as hemophilia gene therapy and will be activated by furin and increases proportion of activated form of FVII.

p-679
RELATIONSHIP BETWEEN THE GLUTATHIONE S-TRANSFERASE T1 POLYMORPHISM AND INTELLIGENCE QUOTIENT IN HIGH SCHOOL STUDENTS

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Glutathione S-transferase (GST), the first enzyme in the mercapturic acid pathway, catalyzes the nucleophilic addition of the thiol of GSH to electrophilic acceptors. It is important for detoxification of xenobiotics and for protection of tissues from oxidative damage. Deletion in GST genes might be having some effects on several tissues such as brain. The primary aim of this study was to investigate the impact of the glutathione S-transferase, subclasse θ (GSTT1) on intelligence quotient. Subjects were 120 high school students of Fereydoonshahr (Isfahan province). The GSTT1 genotype was determined using a polymerase chain reaction-based method. Intelligence quotient (IQ) was measured by Ravan test. The linear regression method was applied. There is significant association between GSTT1 genotype and IQ ($t = 2.689$, $P = 0.008$). Our study suggests that GSTT1 has a measurable impact on IQ.

p-680
ISOLATION, INDUCTION OF NEURAL DIFFERENTIATION AND GENE EXPRESSION PROFILING OF NCAM, L1, N-CADHERIN AND NINJ1 DURING THE COURSE OF DIFFERENTIATION OF MOUSE NEURAL STEM CELLS

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Introduction: It has long been thought that functional regeneration of the injured central nervous system (CNS) is impossible. Accordingly, during mammalian neural development, most neuronal production (neurogenesis) occurs in the embryonic stage. However, recent findings indicate that neurogenesis continues in several regions of CNS including olfactory bulb, hippocampus, and dentate gyrus of adult mammals. Isolation and characterizing the nature of cells responsible for neurogenesis, neural stem cells (NSCs), in adult CNS would have a potential application for cell-based therapies of neurodegenerative diseases. The aim of this study is to isolate mouse neural stem cells, induce their neural differentiation and to profile the expression of some important genes during the course of neural differentiation. Material and Methods: Neural stem cells were isolated from 3-month-old C57 mice by means of their ability to grow in NSC media and producing neurospheres. NSC media supplemented with 1% serum was employed to induce neural differentiation. Total RNA for gene expression profiling extracted from differentiated and undifferentiated cells. To evaluate how closely differentiated cells mimic the real neural cells, we studied the expression profile of NCAM, N-Cadherin, L1 and Ninj1 during the course of neural differentiation of the cells. Results: Mouse NSCs isolated from SVZ in lateral ventricles produce neurospheres, which are evident 10-11 days after culturing. Multiple passaging confirmed the stemness nature of the cells. In addition, isolated NSCs were able to differentiate into neural-like phenotype when cultured in NSC media having 1% fetal calf serum. RT-PCR results revealed that the expression of some neuronal genes is induced during differentiation. Discussion: Our findings confirm that a subset of cells in lateral ventricle SVZ in mouse CNS have stemness nature and are able to differentiate into neural-like cells in vitro. The finding suggests that these cells are a suitable candidate for cell-based therapy of brain and spinal cord injuries.

p-681
HELICOBACTER PYLORI CAGA STATUS AND S AND M ALLELES OF VACA IN ISOLATES FROM INDIVIDUALS WITH A VARIETY OF H. PYLORI-ASSOCIATED GASTRIC DISEASE

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Colonization of the mucosa of the stomach and the duodenum by Helicobacter pylori is the major cause of acute and chronic gastroduodenal pathologies in humans. The vacuolating cytotoxin and the cytotoxin-associated protein, encoded by vacA and cagA respectively are important virulence determinants of H. pylori. However the relationship between genotypes of both cagA and vacA and resultant gastroduodenal pathology is unclear. The objective of this study was to correlate vacA genotype and cagA status with gastroduodenal pathology. Seventy-seven Iranian H. pylori strains from dyspeptic patients (35 with duodenal ulcer, 13 with gastric ulcer, and 29 with chronic gastritis) were studied for differences in the vacA and cagA genes and their relationship to the clinical outcome. By PCR fifty-five of 77 strains (71%) had the vacA signal sequence genotype s1 and only 22 (29%) had the type s2. After primer modification the vacA middle-region types m1 and m2 were detected in 31 (40%) and 46 (60%) strains, respectively. The combination of s1-m2 (24 [31%]) and s1-m1 (31 [40%]) and s2-m2 (22 [29%]) had similar percentage. No strain with the combination s2-m1 was found. Thirty-eight (79%) of 48 patients with peptic ulcer harbored type s1 strains, in contrast to 16 (55%) of 29 patients with gastritis. Thus the presence of the vacA s1 genotype was associated with peptic ulcer disease ($P = .04$). The vacA genotype s1 was significantly associated wit the presence of cagA ($P = .0001$). The cagA gene was detectable in 52 (68%) of 77 H. pylori strains and presence of cagA gene was associated with peptic ulcer disease ($P = .01$). From our study, we conclude that there is a significant association of the cagA gene and vacA s1 signal sequence with gastroduodenal ulcer disease. The relationship of the various other vacA genotypes to gastroduodenal ulcer disease is less clear.

p-682
GENETIC POLYMORPHISM OF GLUTATHIONE S-TRANSFERASE M1 AND INTELLIGENCE

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Glutathione S-transferases M1 (GSTM1) is an important phase II enzymes involved in the detoxification of many electrophilic compounds by conjugating them to glutathione. The GSTM1-0 allele (null allele) represents a deletion of the GSTM1 gene. Homozygosity of the null allele results in a loss of enzymatic activity (null genotype). Deletion in GST genes might be having some effects on several tissues such as brain. We have investigated the effect of the glutathione S-transferase, subclass μ (GSTM1) on intelligence quotient. Subjects were 126 high school students (male=44, female=82) of Fereydoonshahr (Isfahan province). The GSTM1 genotype was determined using a polymerase chain reaction-based method. Intelligence quotient (IQ) was measured by using Ravan test. The Independent-Samples T Test method was applied. There is not significant association between GSTM1 genotype and IQ (t = 1.468, P = 0.145, df =124). Our study suggests that polymorphism of GSTM1 does not have a measurable impact on IQ.

p-683
RELATIONSHIP BETWEEN MUTATIONS OF MITOCHONDRIAL DNA ND1 GENE AND TYPE 2 DIABETES

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Type 2 diabetes is a genetically heterogeneous disease consisting of several sub-groups with various combinations of susceptibility genes. Identification of genes that predispose individuals to develop Type 2 diabetes will facilitate early diagnosis, effective treatment and intervention. Recent studies have indicated some mutations in mitochondrial DNA ND1 gene are related to diabetes mellitus. In this study PCR restriction fragment length polymorphism (PCR-RFLP) was used for screening 4 spots (nt3243, nt3316, nt3394, nt3426) of mitochondrial DNA to explore relationship between ND1 gene mutations and type 2 diabetes mellitus. We didn't find any previously reported mutations in 200 diabetic and 100 control subjects. According to our findings we conclude that there isn't any relationship between type 2 diabetes mellitus and ND1 gene mutations in Guilan population.

p-684
DIFFERENTIAL EXPRESSION OF NEUROTROPHINS AND THEIR RECEPTORS DURING REGENERATION OF DISSECTED SCIATIC NERVE

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Since adult mammalian nervous system has a very limited capacity to replace neurons lost after lesion, understanding the mechanisms regulating their survival or elimination is of special intrest. Neurotrophin family (NGF, BDNF, NT3) are target-derived factors, capable of promoting both neuronal survival and death. These proteins function by means of two types of specific (TrkA, TrkB, TrkC) and common (p75NTR) receptors. The aim of the present study was to monitor the changes in the expression of NTs and their receptors during the course of regeneration of injured nerve and spinal cord. Right sciatic nerve of adult male Wistar rats was transected distal to the obturator tendon. At the indicated times after axotomy the animals were sacrificed and the proximal and distal segments of the sciatic nerve, and the lumbosacral part of the spinal cord were collected. Total RNA was extracted from each sample and the expressions of interested genes were studied by semi-quantitative RT-PCR. We found that NGF and its receptor (TrkA) are induced in proximal and distal segment of the sciatic nerve after transection. Expression of most other genes declined in the initial hours after transection, followed by an increase at 3-7 days after surgery, then declined to the primary level. Expression of a subset of genes, such as BDNF, NT3 and TrkC increased in distal segment of dissected sciatic nerve. In contrast, TrkB receptor showed down regulation in distal segment. Our findings reveal that the expression of NTs and their receptors are differentially regulated during the regeneration of damaged neuronal tissues and suggest that NTs could play a role in neuronal survival and death during the course of regeneration. The data also suggest that manipulation in the expression pattern of NTs and their receptors might affect the success of regeneration of injured neural tissues.

p-685
INTRACTION OF IVSI-5 MUTATION AND HAPLOTYPES IN BETA-THALASSEMIA

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Background and Objectives: The hemoglobin disorders are a group of autosomal recessive disorders causes anemia with different severities. β-thalassemia is one of the commonest genetic disorders characterized by either absence or reduced β-globin chains synthesis. One of the commonest mutations causing β-thalassemia especially in south of Iran is IVSI-5(G>C). There are numerous polymorphic base substitutions within the β -glibin-gene cluster. They produce RFLPs, which are combined in a limited number of haplotypes that are in linkage disequilibrium with β -globin-gene mutations. The aim of this study was to analyze the relationship between β-globin cluster haplotype and IVSI-5 mutation. Materials and Methods: After obtaining informal consent, DNA was extracted from 5 ml of peripheral blood of β-thalassemia carriers referred from primary health care centers. PCR-ARMS was performed for detecting the common mutations. Haplotype analysis was done by using PCR-RFLP in three different sites. Polymorphisms included: GγHindIII, 3'ψβHindII, AvaII/β. Polymorphisms detection were investigated by digesting PCR products by apropriate

restriction enzyme. Results and Discussion: In this study, 41 β- thalassemia carriers and their parents with IVSI-5(G>C) mutation were studied. Total of 41 case with IVSI-5(G>C), 68.69% had the pattern type I and type V (- - +). Tthe remaining cases (38.40%) had haplotype VII (- - -). Rest of the cases didn't have informative pattern. Our study showed that based on haplotype analysis, non-random association of polymorphic restriction sites in the β-gene cluster occurs within the common mutations in β-globin gene like IVSI-5.

p-686
THE ROLE OF ALPHA-GLOBIN GENE DELETIONS IN THALASSEMIA INTERMEDIA

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Thalassemia intermedia is a term used to define a group of patients with beta thalassemia in whom the clinical severity of the disease is somewhere between the mild symptoms of beta thalassemia trait and the severe manifestations of beta thalassemia major. The aim of this study was to find the role of α-globin gene deletions (secondary modifier) in the molecular pathogenesis of b -thalassemia intermedia. The diagnosis of thalassemia intermedia was made based on the patient hemoglobin (Hb) at satisfactory level between 7-10 g/dL at the time of diagnosis without the need for regular blood transfusions. After obtaining informed consent, 10 milliliters of peripheral blood from 45 thalassemia intermedia patients were collected in EDTA-containing tubes and stored at 4˚C. The genomic DNA extraction was performed by salting out method. In addition to finding mutations in b -globin gene, we examined some widespread a -globin gene deletions in Iran such as -α3.7, -α4.2, -α20.5 and -- MED by Multiplex Gap PCR method. After that we run electrophoresis on 1% agaros gel and their results were interpreted. In this study, we found 5 cases who had deletion in a -globin gene, probably ameliorating the phenotype of thalassemia in these patients. Three patients had – α3.7 deletion in heterozygous form, one -α20.5 deletion and one -- MED deletion. These patients had homozygous or compound heterozygous b ° mutations in b -globin gene. It seems different factors are involved in b -globin gene mutations like β+ and β ++ that results in HbF increase or are effective in pathogenesis of thalassemia intermedia in Iran.

p-687
PHYLOGENETIC RELATIONSHIP OF TWO MULLET SPECIES IN NORTH OF IRAN

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Introduction: The genetic differentiation and phylogenetic relationships between two species of the Mugilidae family (Liza aurata & Liza saliens) were investigated at the mtDNA sequencing on samples taken from Mazandaran (8 individuals) and Gilan (8 individuals) region in southern part of Caspian Sea. Method: DNA was extracted from muscle tissue, by phenol-chlorophorm method. PCR primers were designed by D-Loop sequence of gene bank. Results: Using a pair of specific primers a fragment of 332 bp long was amplified from D-Loop of mtDNA. Products of amplification were recognizd by electrophoresis on 1% agarose gel stained with ethidium bromide. For sequencing of genomic DNA, PCR products were extracted from agarose gel with purification kit. Purified DNA sent to a company for sequencing. Data analysis was performed using the software programes and phylogenetic trees were drawn to compare the species. Conclusion: According to phylogenetic trees, species were clustered in two separate groups, the first one containing Liza aurata and the other Liza saliens .

p-688
THE ROLE OF GαQ ON β-CATENIN EXPRESSION BY USING A PEPTIDE THAT INHIBITS GαQ SPECIFICALLY

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Wnt signaling is an important pathway, which plays a key role in various cellular and developmental events such as proliferation, differentiation, migration and polarity of cell. One of the components of this pathway is the multifunctional protein, β-catenin. This protein has an important role in cell-cell adhesion via E-cadherin and also (as a member of wnt signaling pathway) regulates expression of some important genes such as cyclin D1 and c-myc. Deregulation of wnt signaling occurs during formation of several human cancers and therefore this pathway has been considered as a target for cancer prevention and therapy. In this project, the relationship between the Gαq class of G-proteins and β-catenin expression has been investigated. We have made a minigene construct encoding an inhibitor of Gaq and transfected it into 293T cells by calcium phosphate method. The expression and function of β-Catenin in the presence and absence of the Gaq inhibitory peptide will be discussed in the poster presentation.

p-689
QUANTIFICATION OF CEA MARKER FOR MICROMETASTASES DETECTION IN GASTRIC CANCER BY REAL-TIME PCR

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Gastric carcinoma is one of the most prevalent cancer types in the world today. Gastric adenocarsinoma is the first leading fatal malignancy in Iran. About 25 per 100,000 Iranians are affected with gastric cancer each year. Despite advances in novel therapeutics approaches for gastric cancer patient, tumor dissemination via blood stream to distant organ is still the major cause of death. Therefore, there is urgent need to establish sensitive methods for early detection of disseminated tumor cells in peripheral blood of gastric cancer patients. In the present study we used CEA as a tumor marker and GAPDH as an internal control to detect and quantify disseminated tumor cells in peripheral blood specimen of affected individuals. Total RNA was extracted from AGS cell line and CEA and GAPDH fragments were generated by reverse transcription. The amplified fragments were cloned into T/A vector separately. Double cloning of these genes has done into one T/A vector. Serial dilution of this plasmid will be used as standard curve, each containing a known amount of input copy number. Total RNA was extracted from peripheral blood specimens of about 20 patients. cDNA of the specimens were synthesized by reverse transcription. We set up Real-time PCR for CEA and GAPDH. Finally using Real-Time PCR we will determine wether there are disseminated tumor cells in the peripheral blood specimens of these patients.

p-690
STUDY OF MUTATION OF EXON 9 OF P53 GENE IN HISTOPATHOLOGIC BLADDER SPECIMENS EMBEDDED IN PARAFFIN IN KERMANSHAH BY NON-RADIOACTIVE PCR-SSCP METHOD

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Urinary bladder cancer is the fourth commonly diagnosed malignancy in men and the tenth commonly diagnosed malignancy in women worldwide. Bladder cancer is associated with smoking and occupational exposures and successful treatment depends on the early detection and specific diagnostic approaches. Missense mutations in the p53 tumor suppressor gene (TP53) are the most common somatic mutations identified among the cancers and are often CpG dinucleotide G:C-A:T transitions that can occur spontaneously and account for almost 25% of p53 mutations in bladder cancers. According to this information and rather high frequency of bladder cancer in Kermanshah, thirty human paraffin embedded bladder cancer specimens were obtained. They were reviewed by a pathologist, and manually microdissected if they included a substantial amount of neoplastic tissue. Tumor samples were analyzed to detect TP53 exon9 gene mutations, with non-radioactive PCR-SSCP technique followed by standard silver staining. SSCP-PCR was performed using internal nested primers and an aliquot of each PCR product as a template. Analyzing nondenaturing polyacrylamide gel electrophoresis gave following results: Mutations were seen in 2 cases (6.7%) that were classified as p53-positive and there were not mutations in 28 cases (93.3%) that were classified as p53-negative. Results from the above analyses indicate that TP53 gene exon 9 mutations are correlated with bladder cancer in Kermanshah population.

p-691
DETECTION OF CYTOKERATIN 20 mRNA IN THE PERIPHERAL BLOOD OF PATIENTS WITH COLORECTAL CANCER BY REAL-TIME REVERSE TRANSCRIPTASE POLYMERAS CHAIN REACTION

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Colorectal carcinoma (CRC) is the third most common cause of cancer related deaths in the world, which involves epithelial tissue of colon and rectum. Despite advances in novel therapeutic approaches for colorectal cancer patients, tumor cells dissemination via the bloodstream or lymphatic circulation to distant organ is still the major cause of death. Therefore, there is urgent need to establish sensitive methods for early detection of disseminated tumor cells or micrometastasis in peripheral blood or lymphatic circulation of CRC patients. Detection of circulating cancer cells is a useful indicator for the risk of recurrence of advanced carcinoma. Cytokeratins (CKs), which are major constituents of the epithelial cytoskeleton, are abundantly expressed by epithelial cells. Normal, epithelial cells are not capable of migrating outside their original host organ. Thus the presence of extrinsic epithelial cells in peripheral blood indicates the malignant nature of these cells. The aim of the present study is to evaluate the feasibility of real-time reverse transcriptase–polymerase chain reaction (RT-PCR) detection of free cancer cells in the peripheral blood as a prognostic indicator for patients with colorectal cancer. For this purpose, we are quantifying CK20 mRNA levels and CK20/GAPDH mRNA ratios using a real-time PCR system with fluorescent hybridization probes in the blood samples of Iranian patients. As free RNA is degraded rapidly by RNAse in blood, the detection of mRNA is equivalent to the detection of viable cells. Final results will be reported in the congress.

p-692
INVESTIGATING THE MUTATION OF BRCA1 GENE EXONS NUMBER 5,11A AND 11B IN HISTOPATHOLOGIC BREAST PARAFFIN EMBEDDED TISSUE SPECIMENS BY NON-RADIOACTIVE PCR-SSCP METHOD IN KERMANSHAH

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Breast cancer is a malignant proliferation of epithelial cells lining the ducts or lobules of breast. It is the most common malignancy in women, accounting for one third of all cancers worldwide. Despite the high incidence of sporadic cases, incidence of familial breast cancer is low. Considering the daily rise of breast cancer incidence in various parts of Iran,

including Kermanshah, a molecular investigation seems to be necessary. It is indicated that the most human cancers are caused by an abnormal activity of proto-oncogenes and/or inactivation of tumor suppressor genes. In this study we have used a sensitive and non-radioactive PCR-SSCP technique to detect BRCA1 mutations. Thirty human breast paraffin embedded specimens were obtained from patients with sporadic breast cancer. They were reviewed by a pathologist, and manually microdissected if they included a substantial amount of neoplastic tissue. Tumor samples were analyzed to detect BRCA1 exon 5, exon 11A (300bp) and exon 11B (296bp) gene mutations, with non-radioactive PCR-SSCP technique followed by standard silver staining. Analyzing nondenaturing polyacrylamide gel electrophoresis gave us following results: Mutations were seen in 6 cases (20%); 4 cases (13.3%) in exon 5, no case in exon 11A and 2 cases (6.7%) in exon 11B. The results of above-mentioned analyses revealed that mutations in exon 5 and exon 11B contribute to the development of sporadic breast cancer in Kermanshah.

p-693
EVALUATION OF TWO MICROSATELLITE MARKERS RM004 AND ILSTS002 IN LORI-BAKHTIARI STRAIN SHEEP

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Microsatellites are simple sequences of DNA consisting of short tandem repeats and according to its size are highly polymorphic. For this reason, they are used as tools for recognizing the identity and also in the study of species. Butso far, no special function has been known for them at genome level. The aim of this study is to examine the presence of two microsatellite markers RM004 and ILSTS002 in the genome of Lori-Bakhtiari strain of sheeps and to determine polymorphism in this species. Blood samples collected from 50 Lori-Bakhtiari sheeps and DNA was extracted by modifed salting out procedure. The polymerase chain reaction was used for amplification of these markers with the specific primers. The PCR products were resolved on a non-denaturing 10% polyacrylamide gel by electrophoresis. For RM004, in 26% of the examined sheep the 100 bp DNA fragment was amplified, 44% of sheep showed the 150 bp fragment and in 30% of them both of 100 bp and 150 bp fragments were identified. About ILSTS002 marker, all of the examined samples were homozygus and showed 120 bp fragments. Since the RM004 marker in all of the studied sheep was seen only as two kinds of DNA fragments of 100 and 150 bp and ILsts002 marker only with a 120 bp size, It seems these two markers can be used as an applicable marker for identifying Lori-Bakhtiari sheep.

O-694
CLONING OF THE INFLUENZA A (H9N2) VIRUS M2 GENE IN PAED4 EXPRESSION SYSTEM AS A CANDIDATE GENE FOR UNIVERSAL RECOMBINANT VACCINE

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Influenza is an ancient disease that has infected humans in irregular intervals throughout past history. Most recently, in 1997 and 2003, limited outbreaks caused by a new influenza A virus subtype H5N1 that was directly transmitted from birds to humans occurred in the Hong King, a Special Administrative Region of China. Influenza M2 protein is highly conserved across influenza A virus subtypes which was confirmed by M2 gene sequencing and comprising obtained sequencing data with Genbank data in our last study and it was proved (by other researchers) that vaccines based on conserved antigens would not require prediction of which strains would circulate during a certain season. In order to develop a broad-spectrum protection against different influenza virus strains or variants, some recent studies have been aimed at M2 protein of the influenza A virus. In this study, open reading frame of M2 gene was amplified by two phase RT-PCR using specific primers and pfu DNA polymerase, cloned in pAED4 expression vector in Ecoli (XL1blue). Cloned M2 gene was confirmed by PCR and restriction enzymes pattern using specific primers and EcoR1 and NdeI enzymes, respectively. Cloned DNA fragments were sent for sequencing to check the correct ORF and direct origin of the cloned M2 gene. Currently expression of M2 gene in BL21 is going on. The expressed protein will be analyzed on SDS-PAGE and confirmed by Western blot. Further study for immunogenosity reaction of M2 protein and cross-reactivity to all influenza A subtypes are needed.

p-695
STUDY OF ASSOCIATION BETWEEN -67 A/T POLYMORPHISM IN CORE PROMOTER OF SLC6A3 GENE (DAT1) AND SCHIZOPHRENIA

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According to the dopamine (DA) hypothesis of schizophrenia, schizophrenia is associated with increased activity in dopaminergic neurons. Dysfunction of central dopaminergic neurotransmission has been suggested to play an important role in the etiology of schizophrenia. The Na^{+}-Cl^{-} dependent dopamine transporter (DAT1) is a central regulator of the time and course and synaptic concentration of released dopamine by rapid reuptake of dopamine into synaptic terminals and mediating synaptic reaccumulation of dopamine. In this study we sought to determine the possible association between the SLC6A3 gene (DAT1) core promoter diallelic polymorphic site -67 A/T and schizophrenia in Ahwaz hospitals. Fifty unrelated male patient with schizophrenia were recruited for the study. Also fifty unrelated male controls were randomly selected as control group. Subsequently the allele and genotype frequencies of the polymorphism in two groups were studied. The genotype frequencies in the patients group were as follows: AA: 68% AT: 24% TT: 8% versus the genotype frequencies in the control group were: AA: 78% AT: 10% TT:

12% χ2 test was used for analysis of data. According to this data there is no association between DAT1 and schizophrenia.

p-696
PHYLOGENETIC RELATIONSHIP OF SHIGELLA STRAINS ISOLATED FROM CLINICAL SPECIMENS BASED UPON THE 16S rRNA GENE SEQUENCE

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Background: Shigella is one of the most important agents of acute diarrhea worldwide. Identification of Shigella isolates from each other is difficult. Some reports indicate that study of 16S rRNA is a useful method for this purpose. Based on our knowledge, it is the first report to differentiate Shigella species in Iran by 16S rRNA. Objectives: The aim of this study was to compare homology of Iranian strains with strains from other countries and to introduce a rapid and reliable method to identify Shigella species. Material and Methods: Twenty Shigella isolates from diarrheal patients were studied. They were cultured on XLD and MacConkey agar. Biochemical (IMViC) and serotyping tests were performed to distinguish Shigella strains. DNA was extracted by phenol-chloroform method. Complete gene of 16S rRNA (1542 bp) was amplified by PCR, and then was sequenced by ABI 3130X. We used CLUSTALW software to align the 16S rRNA nucleotide sequences. A neighbor-joining analysis was performed to reconstruct phylogenetic tree with the MEGA 3.1 software . Results: All isolates had 99% similarity. The similarity between S.flexneri and S.dysenteriae was 99.9% and between S.sonnei and S.boydii was 99.8%. The homology between specimens of S.flexneri, S.boydii, S.dysenteriae and S.sonnei were 99.8%, 99.9%, 99.9% and 100.0%, respectively.

p-697
ASSOCIATION BETWEEN 4A/B VNTR AND GLU298ASP POLYMORPHISMS OF ENDOTHELIAL NITRIC OXIDE SYNTHASE GENE AND CORONARY ARTERY DISEASE

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Endo-derived nitric oxide (NO) is synthesized from L-arginine by endothelium nitric oxide synthase (eNOS) encoded by the NOS3 gene on chromosome7. Since reduced NO synthesis in endothelial cells has been implicated in the development of coronary atherosclerosis; we hypothesized that polymorphisms of eNOS gene may be associated with increased susceptibility of coronary artery disease (CAD). We studied the 4a/b VNTR in intron 4 and G298T in exon 7 (Glu298Asp) polymorphisms of eNOS gene in 241 unrelated CAD patients with positive coronary angiograms (>50% stenosis affected at least one coronary vessel) in Shahid Rajaee Heart Hospital and 261 age matched control subjects without a history of symptomatic CAD. The eNOS gene polymorphisms were analyzed by Polymerase Chain Reaction and RFLP. Lipid profile and other risk factors were also determined. The genotype frequencies for eNOS4a/b and Glu298Asp polymorphisms were significantly different between CAD patients and controls (P=0.041 and P=0.003). Also The frequencies of the allele for eNOS4a/b polymorphism and Asp allele for Glu298Asp in exon7 were different significantly between two groups (P= 0.013, odds ratio= 1.84 and P= 0.001, odds ratio= 1.6). Plasma lipids, except HDL-C were also significantly increased in CAD groups. Though the genotype frequencies for 4a/b VNTR in intron 4, Glu298Asp polymorphisms and Asp alleles frequencies were differed significantly between the CAD patients and controls, but this polymorphisms were not identified as independent risk factors of CAD.

p-698
THE EFFECT OF ALA12 ALLELE OF THE PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-2 (PPARγ2) GENE ON BMI IN IRANIAN DIABETIC AND OBESE SUBJECTS

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Objective: Peroxisome Proliferator Activated Receptor γ2 (PPARγ2) is a nuclear receptor that regulates adipocyte differentiation, lipid metabolism and insulin sensitivity. The aim of this study was to evaluate the effect of the Ala12 allele of PPARγ2 gene on BMI in Iranian Diabetic and Obese Subjects. Methods: The genomic DNAs of the 312 subjects including individuals with type II diabetes and with obesity were extracted. Pro12Ala polymorphism was detected by Polymerase Chain Reaction–Restriction Fragment Length Polymorphism (PCR-RFLP) analysis. Results: Frequencies of Ala in obese subjects (q Ala=0.166) were significantly different from those control subjects (q Ala = 0.089) OR95%CI 2.358 (1.101-5.05) (pvalue=0.025). In contrast, no significant association was seen between the Pro12Ala polymorphism and type II diabetes OR 95%CI 0.652 (0.261-1.628). In all subjects, the Ala carriers had higher BMI compared to the common allele. Conclusion: Our results showed that Pro12Ala polymorphism in PPARγ2 gene is associated with obesity in Iranian subjects and presence of the Ala allele associated with higher BMI.

p-699
DISTRIBUTION OF ALLELIC VARIANTS OF FUNCTIONAL C3435T POLYMORPHISM OF DRUG TRANSPORTER MDR1 GENE IN A SAMPLE OF IRANIAN POPULATION

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P-glycorprotein (P-gp), the protein product of MDR1 gene, is an important factor regulating the bioavailibity of many therapeutic agents. Recently, the C3435T polymorphism of MDR1 was correlated to altered expression and function of P-gp in normal tissues. In this study, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay was applied to assess C3435T MDR1 polymorphism in 137 healthy individuals of Khorasan origin from the population of Iran.The detected genotype variant frequencies were as follows: TC in 56.2%, TT in 26.3% and CC in 17.5% of the investigated subjects; the T allele frequency was 54.4%. The results of this study give basis for large-scale investigations of MDR1 C3435T genotype-phenotype correlation in Iranian population that may be useful for designing individual-specific therapy for diseases like cancer, HIV-1 infection and some other dideases.

p-700
THE HAPLOTYPE OF β-GLOBIN CLUSTER IN FR36/37 MUTATION OF β-GLOBIN GENE

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Objective: The clinical presentation of β-thalassemia is highly variable, ranging from mild to severe anemia. It seems thalassemia is the commonest monogenic disorder in Iran. Fr36/37 is one of the common point mutations in the country with high prevalence in the southern provinces. This mutation causes a β0 phenotype and occurs at exon 2 of β-globin gene. The aim of this study was to find the relationship between Fr36/37 mutation and β-globin cluster haplotype among 37 β-thalassemia alleles in Iranian carriers of β-thalassemia. Materials & Methods: Genomic DNA was extracted from 5 ml of peripheral blood of Iranian carriers of β-thalassemia referred from Primary Health Care (PHC) centers. Fr36/37 mutation was tested by amplification refractory mutation system (ARMS) PCR. For haplotype analysis 3 different sites in β–globin cluster (Gy--HindIII, 3' βHincII, β-AvaII) were analysed using PCR-RFLP. Results and Discussion: Fr36/37 mutation was found in 37 β-thalassrmia carriers and their families. Using PCR-RFLP it was shown that all of the individuals have haplotypes I and V (- - +). This study shows that these haplotypes are in linkage disequilibrium with Fr36/37 mutation. These haplotypes probably have some modifier impacts on the phenotype of β-thalassrmia.

p-701
DETECTION OF HTLV-1 AND HHV-8 SEQUENCES IN KAPOSI SARCOMA

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Kaposis sarcoma (KS) is a common malignant tumor in immuno compromised individuals and in organ transplant recipients due to immunosuppressive drugs. The correlation of lymphotropic viruses such as HHV-8 and HTLV-1 to KS is currently unde investigation. These viruses might be the risk factors or the primary cause of KS. In this study we selected 40 paraffin embedded skin biopsies with KS diagnosis.Theses diagnoses were reconfirmed by careful microscopic examination. Five micron thick sections of the blocks were prepared and after deparaffinization, their DNA was extracted. Using env, tax and pol specific primers and ORF26 and ORFK9-1 specific primers the HTLV-1 and the HHV-8 genomes were amplified, respectively. Our results indicate that none of the KS tissues were infected with HTLV-1, however we detected the presence of HHV-8 genome in more than 90% of the specimens. HHV-8 also known as KS might be reactivated under conditions of immunodefficiency in the host, and HTLV-1 infection leads to immunodefficiency and infected individuals have a propensity to opportunistic infections and malignancies. But our data showed no significant association between HTLV-1 infection and KS. It is thus considerd that HTLV-1 has no relation to the progression or immune condition of KS pations.

p-702
POLYMORPHISM OF CALPASTATIN GENE IN KERMANIAN SHEEP USING PCR-RFLP

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To study the calpastatin genotype and allele frequencies in kermanian sheep breeds, blood samples were collected from 100 kermanian sheep breeds. DNA samples were extracted from whole blood and the polymorphism was detected by RFLP method at a 622 bp fragment. Agaroz gel and ethidium bromid were used for the fragment observations. The Allelic and genotypic frequencies of M, N and MN, MM, NN in kermanian sheep breeds were 0.79, .21, 20, 69, 11, respectively. The Chi-square test results ($P \leq 0.05$) revealed deviation from Hardy-Weinberg equilibrium. Average heterozygosity in kermanian breeds were 20.

p-703
DETERMINATION OF KAPPA CASEIN POLYMORPHISM IN INDIGENOUS AND FOREIGN KERMAN CATTLE

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The genotypes and allele frequencies for k-casein variants A and B in indigenous and foreign Kermanian cattle were determined. In this study blood samples were collected from 55 indigenous and 50 foreign cattle. Genomic DNA isolated from blood. The polymerase chain reaction was used to amplify a 228-bp fragment from the K-casein gene. Using PCR-RFLP with two primers SGO and SGE and two restriction endonucleases (Hinf I and Hind III), the Distribution of k-casein genotypes in indigenous Kermanian

cattle were 36 AA, 6 AB, and 11 BB and in foreign Kermanian cattle were 32 AA, 11 AB, and 7 BB. The frequency of B and A alleles in the population studied were 0.32 and 0.68, respectively. The frequency of B and A alleles in the population studied were 0.25 and, 0.75 respectively. In contrast to a previous study, the frequency of B allele has been increased. It is probably because of selecting B allele in breeding programs. The Chi-square test results ($P \leq 0.05$) revealed deviation from Hardy-Weinberg equilibrium of genotypes. The two Alleles A (Thr136, Asp148) and B (Ile136, Ala148) have a great importance in production traits, such as milk yield, protein, fat contents and especially cheese making properties. Because of the important role of the B variant on increasing in milk protein and fat content, as well as cheese production, its effect is known to be economically important, and it is considered to be included in dairy breeding programs.

p-704
CYSTIC FIBROSIS: A FREQUENT DISEASE WITH HETEROGENOUS MUTATION SPECTRUM IN IRAN

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Background: Cystic fibrosis (CF) is the most common autosomal recessive disorder in European populations with about 1:25 carrier frequency and regional variation. Since the identification of the cystic fibrosis transmembrane regulator (CFTR) gene, over 1000 mutations have been reported in which ΔF508 is the most frequent. However for non-European populations, the incidence of CF and the spectrum of mutations that are responsible for the diseasehave not been investigated extensively. A few comprehensive studies are focused on the molecular basis of CF in the Iranian population. In this research we are going to present the results obtained from a 4 year study on the 110 Iranian CF patients including molecular analysis of the CFTR gene. Methods: Mutation detection was performed using ARMS-PCR, SSCP, and Sequencing. Results: Our results suggest that CF is a very heterogenic disease among our population in which about 15 different mutations and polymorphisms have been detected till now. DelF508, G542X, N1303K, G551D and W1282X, the 5 most common mutations in Europeans with the overall frequency of 75%, only contain 23% of CFTR mutant alleles in Iran. The other detected mutations are rare with relative frequencies of lower than 1%. Conclusion: The information provided here on the distribution of CF mutations in Iranian population may assist in the development of more appropriate diagnosis tests in Iran and also it may facilitates the mutation analysis in the neighboring countries.

p-705
MOLECULAR CLONING OF THE PROTEIN 43 TOXOPLASMA GONDII TACHYZOITE

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Toxoplasma gondii is an obligate parasite of cat and other animals are intermediate hosts. It can live in nucleated host's cells. Toxoplasmosis is generally asymptomatic, but often makes a sever problems in immunocompromised patients such as those suffering from AIDS (Acquired Immunodeficiency Syndrome). Over one-third of people around the world are seropositive. If a pregnant woman encounters toxoplasma for the first time, the probability of fetus transfection transplacentally is high and called congenital toxoplasmosis and may lead to blindness, mental retardation or even death. Differentiation between acute and chronic infection is paramount important. In the acquired acute infection, it appeared in tachyzoite form with specific surface antigens and used for serological laboratory diagnostic test. Laboratory diagnosis is usually based on detection of specific antibodies against surface antigens. Therefore the availability of specific antigens is important. In this study the protein P43, a tachyzoite specific antigen, was cloned in a suitable vector as a recombinant proteinproduction. For this purpose, a pair of forward and reverse primers containing SacI and BamHI restriction sites at the 5` ends respectively was designed based on toxoplasma p43 tachyzoite gene. The PCR product cloned in an intermediate vector and recombinant plasmid was digested using these enzymes and subcloned in pGEMEX-1 expression vector.

p-706
MOLECULAR ANALYSIS OF IRANIAN AZERI TURKISH PATIENTS WITH DUCHENNE/ BECKER MUSCULAR DYSTROPHIES

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Duchenne and Becker Muscular Dystrophies are allelic disorders caused by mutations in the X-linked dystrophin gene. DMD is one of the most frequent neuromuscular diseases affecting 1 in 3500 live male births. Deletions in the dystrophin gene represent 65% of mutations in DMD/BMD patients and one third of cases arise from new mutation. We have analyzed DNA from 36 Iranian Azeri Turkish patients with DMD/BMD using the multiplex polymerase chain reaction (M-PCR) to screen for exon deletions within the dystrophin gene. All members of the families (approximately 100 individuals) were studied by using 11 microsatellites located in the interested region. Twenty-five patients (70%) had deletions in at least one of the 26 studied exons. All deletions were clustered in the two known hot-spots regions, and in 92% of cases deletions were detected in the distal region from exon 40 to exon 52. Informative microsatellites were selected for linkage analysis. Applying M-PCR enabled to diagnose 70% of Iranian Azeri Turkish DMD patients harboring deletions. Using highly polymorphic intragenic and flanking markers enabled to identify most of the carriers for the purposes of prenatal diagnosis. In 50% of the males harboring large deletions, intragenic markers from the relevant region were shown to be deleted using multiplex PCR. These markers were studied in the female members of the relevant families and LOH was observed in two of them. It is important to note that in our population, we can first search for deletions of DMD gene in the most frequently deleted exons determined by this study.

p-707
ADENOSINE DEAMINASE GENE POLYMORPHISM IS ASSOCIATED WITH OBESITY IN IRANIAN POPULATION

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Adenosine deaminase is a polymorphic enzyme, which has important role in modulation of insulin bioactivity. It has been suggested that an excess of adenosine A1 receptor activity may contribute to adiposity in type 2 diabetes. Adenosine seems to have a role in facilitating insulin action on the adipocytes. ADA gene polymorphism seems to contribute to the degree of obesity in type 2 diabetes patients. The aim of this study was to examine the role of ADA gene polymorphism in a randomly selected obese subject in Iranian population. A significant increase in the frequency of ADA, AA genotype was observed in obese and also diabetic people with BMI≥ 30 compared to the controls. There was also a significant increase in ADA gene allele A and AA genotype frequency in patients with higher plasma cholesterol level compared to the normal controls. There was also a significant difference when we compared the ADA allele frequency in patients with obesity and higher plasma cholesterol level and patients with obesity and lower plasma cholesterol level. We also examined the association between the frequency of ADA allele and genotype frequencies with triglyceride levels in obese patients and normal controls. We observed a significant increase in the ADA gene AA genotype frequency in obese patients with TG level ≥150 mg/dl compared to the normal controls. Our data indicates the role of ADA in obesity and also its effect on abnormal level of TG and cholesterol in obese people. Also our findings recommend adenosine receptors as important targets for new therapeutic.

p-708
TYPING OF ONCOGENIC HUMAN PAPILLOMAVIRUS (HPV) IN WOMEN WITH CERVICAL CANCER LESIONS BY MULTIPLEX PCR

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It is well established that persistent infection with high-risk human papillomavirus (hr-HPV) is a necessary cause for the development of cervical cancer. Therefore, inclusion of hr-HPV testing by sensitive polymerase chain reaction (PCR) methodology may improve screening of cervical cancer and management of the disease. This investigation was designed to study the prevalence of the four most common hr-HPVs (types 16, 18, 31 and 33) in the archival tissues with cancerous lesions from Iranian Azeri Turkish population. Seventy-eight formalin-fixed paraffin-embedded tissue specimens were tested by using two type-specific multiplex PCRs. In total, 52 (70%) out of 75 amplifiable samples were positive for at least one hr-HPV type. HPV 16 was the most common type among the identified oncogenic types (65%). The presence of multiple infected samples in the studied cases along with rather different circulating HPV types in the studied population will help us to plan more efficient screening and vaccination programs in the future.

p-709
USING MULTI-SAMPLE SLIDES SPOTTED WITH UNIVERSAL PROBES TO DETECT COMMON CYP1B1 MUTATIONS IN PRIMARY OPEN ANGLE GLAUCOMA PATIENTS

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Disease in 70% of Iranian Primary Congenital Glaucoma (PCG) patients is due to mutations in CYP1B. There are four common CYP1B1 mutations in these patients. Although CYP1B1 is commonly associated with PCG, there is evidence that it may also have a role in Primary Open Angle Glaucoma (POAG), a far more common form of glaucoma. We aimed to assess the role of CYP1B1 in POAG patients by screening their DNAs for the common PCG mutations. Screening was done on a microarray platform to test the suitability of multi-sample slides spotted with universal probes. Each slide was divided into 48 cells; each slide could therefore be used to analyze 48 individuals, resulting in significant reduction of slide cost. Each cell was spotted with 30 tag oligonucleotides, in triplicate. By appropriate design of PCR or cDNA synthesis primers, the tags could potentially be used to investigate any genomic sequence variation or the expression of any gene. The slides were therefore universal therefore further costs reduction. Eight of the tag probes were used to assess the genotypes by hybridization with fluorescently labeled products of a modified ARMS-PCR. Homozygous mutant, homozygous wild type, and heterozygous genotypes at all four positions could easily be distinguished. There was perfect reproducibility at the three replicate spottings. Assessed genotypes of seventy-one POAG patients were confirmed in a random sub-sample by RFLP and sequencing. Over 10% of the patients carried CYP1B1 mutations. The results show the significant role of CYP1B1 in POAG, and the suitability of the microarray platform for genotype identification.

p-710
SOMACLONAL VARIATION AMONG SOMATIC – EMBRYO DERIVED PLANTS OF OLEA EUROPAE L.

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Somaclonal variation in plants of Olea europaea L. cv. Kroneiki derived from somatic embryogenesis were examined by randomly amplified polymorphic DNA (RAPD) analysis. Radicle and cotyledon (proximal part) of mature zygotic embryo were cultured in OMc medium supplemented with IBA (5 mg1-1) and 2ip (0.5mgl-1). 21 day calli were subcultured in OM medium. The percentage of somatic embryogenesis in calli obtained from radicles and proximal

parts of cotyledons were 55% and 10%. Regenerated plants were obtained after culturing embryonic calli on OM medium in the presence of BAP (2mgl-1). DNA samples from leaves of mother plant, seedling (germinated from zygotic embryo), plantlets (regenerated from somatic embryos) were subjected to RAPD analysis. 20 arbitrary decamer primers produced polymorphic amplification products. The estimation of genetic similarity coefficient based on RAPD band sharing data indicated that regenerated plants were less than 80% similar to mother plants.

p-711
THE EFFECTS OF GRISEOFULVIN ON THE EXPRESSION OF ATPase-SUBUNIT G GENE IN DERMATOPHYTE PATHOGEN TRICHOPHYTON RUBRUM

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Trichophyton rubrum is one of the anthropophilic dermatophytes with worldwide distribution. This fungus is a common causative agent of tinea cruris, tinea corporis, tinea pedis and tinea manuum. Several properties of this fungus have been studied so far. However, few investigations were carried out in the field of molecular biology of this microorganism. The main goal of this survey was the evaluation of ATPase-subunit G gene expression due to different amounts of griseofulvin in T.rubrum.Serial dilutions of griseofulvin have been prepared and added to culture media. The fungus has been cultured under mentioned conditions as well as control. The control and griseofulvin-treated samples were then microscopically investigated and the total RNA was extracted. RT-PCR was performed for all isolated RNAs for evaluation of gene expression of ATPase-subunit G gene under the griseofulvin condition in T.rubrum. The results of this study indicated that abnormal cell bodies such as shortened and twisted mycelia have been increased in cell cultures with 10 µg/ml of griseofulvin. However, based on quantitative RT-PCR, the gene expression of ATPase-subunit G gene showed to be up regulated in 5 µg/ml of griseofulvin compared to the control as well as to 10 µg/ml of griseofulvin condition.

p-712
QUANTITATIVE DIAGNOSIS OF α-AND β-THALASSEMIA CARRIERS BY MEASURING α/β RATIOS USING REAL-TIME RT-PCR

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α-and β-thalassemia are the most common single gene disorders in Iran. National program for the prevention of thalassemia has been in effect since 1997. Sometimes, the hematological and molecular tests are not informative and differentiation between α and β carriers is not easily possible. In clinical laboratories over 90% of beta-globin gene mutations can be detected by molecular tests. But there are some patients who can hardly be distinguished by routine tests. The aim of this study was relative quantification of α- and β- globin genes expression by real-time RT-PCR. Total RNA was extracted from fresh blood samples using TRI reagent. cDNA was synthesized from 5 µg of total RNA with reverse-transcriptase. Primers and Taq-Man probes for α- and β- globin cDNA and β-actin cDNA using Primer Express software to perform real-time RT-PCR were used. The fluorescent reporters and the quencher for α- and β- globin cDNA were: 6-carboxyfluorescein (FAM) and 6-carboxy-N, N, N'I, N'-tetramethylrhodamine (TAMRA), respectively. β-actin probe was fluorescently labeled with JOE. β-actin was used as housekeeping gene to measure α- and β- globin cDNA copy numbers. Quantitative real-time RT-PCR was performed on ABI 7300. ΔCt was calculated for α-globin/ β-actin, β-globin/β-actin and α- globin/β- globin. Then ΔΔCt was calculated for both of them. In conclusion, our study shows that the quantitative Taqman real-time RT-PCR method can be successfully used to assess the gene expression quantification for α-globin and β-globin genes. This technique could be used for final characterization of ambiguous carriers of thalassemia in the national preventional program.

p-713
EVIDENCE FOR PRESENCE OF A COMMON FOUNDER FOR 35DELG AND 313DEL14 MUTATIONS OF GJB2 IN IRANIAN POPULATION

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Background: Hereditary Autosomal Recessive Non-syndromic Hearing Loss (ARNSHL) is a widely heterogenic disorder, which accounts for approximately 70 percent of all genetically based deafness. 35delG is one of the most frequent causes of ARNSHL in many populations. Founder Effect is one of the leading factors of unbalanced linkage disequilibrium. Material and methods: A PCR-RFLP method was used for genotyping of five different single nucleotide polymorphisms (SNPs) distributed in a region of 98 kbp encompassing coding and flanking regions (3' UTR and 5' URR). Further two microsatellite markers D13S175 and D13S141 were analyzed. Results: Analyzing genotypes of the 35delG and 313del14 homozygous chromosomes revealed that 3-T-C-G-T-5-T, 3-T-C-G-T-5-C are the most prevalent haplotypes, accounting for 41% and 100, respectively. A similar study among normal hearing controls leads to the observation of 35-TC-TT-GG-CC-44-TC as the most common genotype. Significant linkage disequilibrium was observed between both 35delG and 313del14 mutations and polymorphic markers suggesting that these two mutations have been originated from a common founder, separately in Iranian population. Discussion: 35delG has a relatively low occurrence in eastern Asian and particularly eastern neighboring population in Pakistan. In contrast, it has a higher prevalence in Iranian population which is closer to that of Iran's northwestern population in turkey and Caucasians in general. Therefore according to the observed data in the above-mentioned populations, we speculate that 35delG has originated in Iranian population. Also, significant difference between haplotype of small

interval chromosomal region shared among normal and 313del14 chromosomes leads to similar conclusion.

p-714
STUDYING GJB2 GENE AMONG PATIENTS FROM EAST-AZARBAYJAN PROVINCE BY SSCP/HA TECHNIQUE

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Hearing loss (HL) is the most common sensory deficit in humans. Roughly one child in a thousand is born with severe to profound prelingual hearing impairment. Non-syndromic hearing loss (NSHL), responsible for 70% cases of childhood deafness, is a paradigm of genetically high heterogeneity with 85 loci and 39 nuclear disease genes reported so far. Mutations in Cx26 (GJB2), a gene that encodes the gap junction protein connexin 26, are the leading cause of ARNSHL in caucasians. Todate 85 different mutations have been reported in this gene. We assess the contribution of Cx26 gene in ARNSHL in 100 probands from the East Azarbaijan Provience. The first phase was screening for 35delG mutations. Heterozygous (5%) or negative (80%) patiants to this mutation, were tested for other associated mutations in the coding region of Cx26 gene by applying SSCP/HA; a technique with high sensitivity, simplicity and versatility. We detected at least 20 different SSCP patterns in affected families. Overall we detected that the Cx26 related HL in East Azarbaijan was 43%. Revelation of several SSCP patterns, potentialy indicates the high incidence of mutations in Cx26 coding region-causing HL in the population of this province. The molecular knowledge of the biological processes responsible for hearing helps form the basis for early diagnosis and therapy, enable providing patients and their families with improved genetic conseling data, and may even facilitate development of strategies for efficient treatment of this common genetic disorder.

p-715
CHARACTERIZATION OF ENTAMOEBA HISTOLYTICA RECOMBINANT GLUCOSEPHOSPHATE ISOMERASE ENZYME

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Protozoan parasite Entamoeba histolytica is an etiological agent of amebiasis, causing an estimated 50 million cases of amebic colitis, dysentery, and extraintestinal abscesses, resulting in 40,000–100,000 deaths annually. E. histolytica is an amitochondriate protist, which lacks both typical mitochondria and hydrogenosomes and is deficient in all features of aerobic metabolism. E. histolytica produces energy by glycolysis and fermentation under an anaerobic or microaerophilic environment. Glucosephosphate isomerase catalyzes the reversible isomerization reaction between D-fructose-6-phosphate (F-6-P) and D-glucose-6-phosphate (G-6-P) as the second enzyme of the glycolysis pathway. In this study, the glucose-6-phosphate isomerase gene of E. histolytica was cloned in frame with poly-histidine tag (his-tag). The recombinant enzyme was characterized, and its enzyme activity was assayed. The formation of G-6-P from F-6-P in the reverse reaction catalyzed by the recombinant glucosephosphate isomerase was measured by monitoring the reduction of $NADP^+$ spectrophotometrically at 340 nm. The recombinant enzyme was active over a wide pH range between 7.0–10.0 (with optimal pH of 8.0–9.0). Glucosephosphate isomerase activity was inhibited by addition of a monovalent cation (Na^+). The recombinant glucosephosphate isomerase followed the Michaelis–Menten kinetics. Kinetic constants under the standard assay conditions (containing 25 – 300 μM substrates) were determined by Lineweaver-Burk plots. The results demonstrated that the recombinant glucosephosphate isomerase had a Km value of 122 ± 16 μM for F-6-P and a specific activity of 786 ± 59 μmol min^{-1} mg $protein^{-1}$ in the reverse reaction. The Km and specific activity of the recombinant glucosephosphate isomerase of E. histolytica were comparable to that of the other organisms.

p-716
INVESTIGATION OF TNF-α POLYMORPHISM IN G6PD DEFICIENT PATIENTS OF IRAN

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Tumor necrosis factor –alpha (TNF- α) is one of the earliest and key cytokines in inflammatory processes, generating a cytokine cascade including the production of interleukin-1, interleukin-6 and also microbial infection. Several single nucleotide polymorphism (SNP) in the TNF- α gene which may affect the transcription and expression, has been identified. An A to G transition directly affecting TNF- α expression is located at - 308 in promoter region. It has been reported that the position -308 A/G can affect the binding of activator protein 2 (AP-2) in which the presence of A polymorphism may inducing higher expression. The affected G6PD deficient samples were collected from three neighboring provinces of Kerman, Yazd and Khuzestan in central part of Iran. The genetic polymorphism of TNF- α-308 A/G promoter has not been reported in Iranian G6PD deficient populations. The TNF- α-308 A/G promoter genotype was determined by polymerase chain reaction –restriction fragment length polymorphisms analysis in 137 G6PD deficiency patients. Allele frequency of TNF- α-308 A/G promoter for above provinces was similar.

p-717
MICROSATELLITE DNA ALTERATIONS IN SQUAMOUS CELL CARCINOMA OF ESOPHAGUS

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Purpose: Esophageal cancer occurs worldwide with a variable geographical distribution. The incidence rate in developing countries, such as Iran, is about 4-fold higher than that in more developed countries. It can be treated effectively by curative surgery if diagnosed at an early stage. Our aim was to develop a novel molecular approach for Esophagus Squamous Cell Carcinoma (ESCC) detection and an indicator for the prognosis of the patients. In recent years, many researchers have indicated that the alterations of microsatellite DNA are one of the important markers leading to induction of normal cells to undergo immortal and neoplastic transformation. Materials and methods: Tumor and normal margin samples were obtained from 27 patients with ESCC. DNA was extracted and microsatellite alterations were examined using the following markers: D13S260 (13q12.3), D13S267 (13q12.3), D9S171 (9p21), D2S123 (2p) and TP53 (17p13.1). PCR amplified segments were denatured and electrophoresed on denaturing polyacrylamid gels and stained using silver nitrate. The microsatellite alteration (Loss of Heterozogosity and Microsatellite Instability) analysis was performed. Results: Twenty-five of twenty-seven specimens (92.9%) were found to have at least one microsatellite alteration in their tumoral tissue. Loss of Heterozogosity (LOH) was obtained in almost 43% (21/27) of the tumors. Microsatellite instability was seen in 55.5% (15/27) of patients. Highest frequency of LOH and MSI was observed in D13S260 (42.3%) and D5S2501 (41%) markers. Conclusion: Microsatellite alterations can be a valuable tool for detection and for the evaluation of the prognosis of esophageal cancer. Further evaluations of more specimens are required for definitive conclusions.

p-718
EXPRESSION OF DMRT FAMILY GENE HOMOLOGUE AMONG IRANIAN ARTEMIA

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DM related family of transcription factors play important roles in male sex determination, and neural development and are expressed in various tissues and different stages of development among species. Members of this family of transcription factors seem to be conserved among diverse species of metazoans, including arthropods, nematode and vertebrates. The name is abbreviated from Drosophila Doublesex (DSX) and C. elegans Mab-3 genes. The conserved motif, so-called DM domain, exhibits a zinc finger-dependent DNA binding property. The Artemia DM domain encodes a 47 amino acid motif and is highly homologous to amino acid sequence of the known DM domains. In this study, first, using DM domain specific primers and PCR amplification a sequence of Artemia (ADM) was amplified out from genomic DNA of two species of Artemia living in Urmia lake that is A. urmiana and A. parthenogenetica. Once the presence of related gene established, we used an RT-PCR approach to visualize the expression of ADM in different developmental stages of Artemia. RT-PCR analysis indicated genes harboring DM domain was expressed in female gonads of both species of Artemia. The expression of ADM was found to be confined to gonads with whole mount In Situ hybridization, confirming our RT-PCR results.

p-719
DETECTION OF SHIGA TOXIN GENES IN E.COLI O157:H7 BY MULTIPLEX PCR

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Strains of shiga toxin – producing Escherichia coli (STEC) have been associated with outbreaks of diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (HUS) in humans. Most clinical signs of disease arise as consequence of the production of shiga toxin/shiga toxin 1 (Stx/Stx1), shiga toxin 2 (Stx2) or combination of these toxins. A technique has been developed for detection of stx/stx1 and stx2 genes by using the multiplex polymerase chain reaction assay with the incrorporation of mdh gene of E. coli. A total of six primers were used: SFI and SRI, which produce a 199 bp product that serves as an internal positive control; Ka2F and Ka2R, which yield a 381bp fragment of stx2 gene, and Ka1F and Ka1R, which amplify a 622 bp fragment of stx/stx1. The thermal profile, which was preceded by a 5 minute incubation at 95 ^{0} C, for 20 –25 cycles with the following parameters: 95^{0}C 1min, 600C, 1min, 72^{0}C, 1min, and 5 min incubation at 72^{0}C as final extention. PCR amplification products identifying the stx/stx1 and stx2 gene sequences were observed only in E. coli O157:H7 and shigella dysenteriae type1. Template nucleic acid extracted from other gram-negative bacteria was found to be negative. The sensitivity of the PCR procedure for detection of shiga toxin genes was determined to be 2.1 pg/µl of total nucleic acid and 320 cfu/µl.

p-720
ASSOCIATION OF GLUTHATION S-TRANSFERASE OMEGA1 WITH ALZHEIMER DISEASE

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Alzheimer disease (AD) is the most significant cause of dementia in developed countries and is clinically characterized by memory loss of subtle onset followed by a slowly progressive dementia that has a course of several years. The AD has a complex genetic component, with the possible involvement of multiple genes and interaction between genes and environmental factors. About 42% of these genes has been found and 58% are yet unknown. Genome wide-linkage studies suggest the existence of multiple additional genes for AD on several chromosomes. A broad linkage peak encompassing a >50 Mb region between chromosome 10q21 and 10q25 has been implicated as influencing either AD risk or plasma levels of Amyloid ß-42 (which cause amyloid plaques in AD brains). Glutathione S-transferase omega 1 (GSTO1) was located under the linkage peak for AD on chromosome 10. The physiological importance of GSTO1 has not yet been fully elucidated. But it protects from oxidative stress, and has effect on Interleukin-1ß Posttranslational

Processing. IL-1ß is accompanied by major changes to the intracellular ionic environment, activation of caspase-1, and cell death. Although some studies showed significant associations with GSTO1 gene and AD, these findings have not been proved, and it remains controversial. We examined association of Ala140Asp (rs4925), SNP 7 polymorphism in exon 4 of GSTO1 with AD. We performed PCR and RFLP technique in a case-control study with 60 sporadic AD cases and 60 age matched controls.

p-721
POLYMORPHISM OF DIACYLGLYCEROL ACYLTRANSFERASE GENE IN DAIRY CATTLE

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The final step of triacylglycerol biosynthesis is catalyzed by acyl CoA: diacylglycerol acyltransferase. DGAT1 was introduced as a functional candidate gene that has significant effect on lactation traits in dairy cattle. Result of study on mice lacking both copies of DGAT1 shows devoid of milk secretion completely. This gene was sequenced in human and cattle on chromosome 8 and 14 respectively. The coding sequence in cattle and human is 1470 bp and organized in 17 exons separated by 16 introns. Dinucleotide substitution (AA to GC) on exon 8 of bovine DGAT1 gene changed lysine to alanine amino acid so DGAT1 have two alleles: allele K, which affectes fat yield, percent of fat and percent of protein and allele A that affects milk yield and protein yield. This gene is a major gene in dairy cattle so information about polymorphism of that is essential for improving milk production traits. This study investigated the polymorphism of the bovine DGAT1 gene on dairy cattle population. Semen samples of 103 bulls were used for this study. A fragment of 411 bp of DGAT1 was amplified by standard PCR and PCR-RFLP method used for genotyping. Frequencies of genotypes KK, KA and AA were 0.59, 0.41 and 0.0, respectively. Allele frequencies of variants K and A were 0.79 and 0.21, respectively. This results show that frequency of allele K is about 4 times more than frequency of allele A. Results of this study could be useful for improving dairy cattle production traits.

p-722
GENETIC MANIPULATION OF HUMAN UMBILICAL CORD VEIN MESENCHYMAL STEM CELLS

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Stem cells are the next frontier in medicine and they are thought to have great therapeutic potential, because they can replace damaged or dysfunctional cells, and also deliver therapeutic genes. For the later purpose, stem cells should be genetically manipulated. Stem cells are defined with two main characteristics, which are self-renewal and multipotency. Currently, the widespread applications are hindered by ethical and technical issues surrounding both embryonic and fetal stem cells. In contrast, cells recovered postnatally from the umbilical cord vein blood and matrix, are readily available and are an inexpensive source of cells that are capable of forming many different cell types. Since human umbilical cord vein stem cells have been recently isolated their different characteristics are now under investigation. Our goal was to isolate mesenchymal stem cells (MSCs) from human umbilical cord vein, to prove their stemness identity and evaluate their potential usage to be genetically manipulated. For this purpose, MSCs were harvested from umbilical cord vein by collagenase treatment and expanded by means of their adhesiveness to plastic surfaces. Flowcytometric assay proved the mesenchymal identity of the isolated cells and showed that they express CD105, CD73 and CD90, and do not express CD54, CD45, CD34, and HLA-DR surface molecules. To prove the stemness identity of the cells, we successfully induced their differentiation toward neural cells by NIM medium and evaluated the expression of several self-renewal genes, including Oct-4, Sox-2, Nanog, Nucleostemin, Bmi-1, Sox-9 and ZFX. We showed that all of the mentioned genes, except Sox-2, were expressed in umbilical cord vein MSCs. Finally, these cells were successfully transfected with pEGFP-C1 plasmid by electroporation method and 24 hours after electroporation, EGFP expression was confirmed using inverted fluorescent microscopy. Our work is in progress to transfect these cells with PCDNA3-hBDNF plasmid, which contains the human BDNF (Brain derived neurotrophic factor) gene. In this study we showed that MSCs derived from the discarded umbilical cord provide a low-cost, pain-free collection method of MSCs. These cells have the potential to be manipulated genetically therefore can offer a new source of cells, for cell-based gene therapy methods.

p-723
THE CLONING AND EXPRESSION OF WILD AND THREE MUTANT TYPES OF κ-HEFUTOXIN

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The venom of scorpion is a rich source of toxins, which affect mainly the ion channels function. They are classified according to their specific target ion channel. Recently, a new, small and weak potassium channel toxin, κ-Hefutoxin, is functionally and structurally characterized from the venom of the scorpion heterometrus fulvipes. NMR studies revealed that κ-Hefutoxin is structurally composed of two parallel helices cross-linked by two disulfide bridges. In order to evaluate the structural effects of disulfide bonds, we report the cloning of the wild as well as three mutant types of κ-Hefutoxin in E.coli cell. In mutant types each pair or all pairs of cystein residues of the wild type disulfide bridges are replaced by serine residue (C4S, C22S and C8S, C18S; C4S, C22S; C8S, C18S). The amplified DNA fragments were inserted in the PET32a

expression plasmid vector between BamH1 & EcoRI restriction sites.

O-724

ASSOCIATION BETWEEN GENETIC DIVERSITY AND CLINICAL OUTCOME OF HELICOBACTER PYLORI ISOLATES

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Genetic diversity of Helicobacter pylori plays an important role in pathogenicity of bacterium. H. pylori containing cagE may be associated with duodenal ulcer and oipA with enhanced IL-8 and increased inflammation. Despite of cagA and vacA there are a few studies about cagE and oipA status of Iranian isolates. The aims of this study were consider of genetic diversity according to presence of cagA, cagE and oipA genes in H. pylori isolates in Tehran, and determination of the association between the cagA, cagE, vacA, oipA genes and clinical outcome. From 180 patients with gastric disorders, 150 gastric biopsies were selected for next steps. The genomic DNA was extracted from biopsy samples by QIAgen kit and stored at 4˚C until PCR amplification. The prevalence of the cagA, cagE, oipA genes was studied in these strains by PCR method. glmM gene was used as a screening test for confirmation of Helicobacter pylori . Out of 150 samples, 115 were positive for glmM which 15 of patient had PUD. Regarding PCR results 90 (78%), 51 (44%), 69 (60%) of the isolates revealed to be cagA, cagE and oipA positive, respectively. In PUD patients 10 (66%) were positive for cagA, 12 (80%) and 9 (60%) were positive for cagE and oipA, respectively. Genetic diversity of H. pylori strains varies from one geographic region to another. Results demonstrated high percentage of cagA positive while all of the patients with cagA gene don't show clinical outcome, suggesting cagA can not be an effective marker of gastritis disorders alone; although cagA was better marker than cagE for presence of cagPAI. High frequency of cagE in PUD patients represents it as a suitable marker for gastritis disorders. We conclude that the presence of oipA besides to cagA and cagE may provide the condition for gastritis disorders.

p-725

SINGLE NUCLEOTIDE POLYMORPHISM AT POSITION -590(C/A) IN IL-4 OF PATIENTS WITH RELAPSING VISCERAL LEISHMANIASIS MIGHT ATTRIBUTE TO THE DRUG RESISTANCE

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Introduction: leishmaniasis can result in a fatal disease, visceral leishmaniasis (VL), or in a self-limiting asymptomatic infection. Cytokines have critical roles in controlling leishmaniasis. The balance between the parasitic-specific T-cell responses derived from T-cell activating cytokines plays an important regulatory role in determining the outcome of Leishmania infections in humans. IL-4 is a Th2 type cytokine playing an important role in suppressing some aspects of T-cell responses. Different polymorphisms in Il-4 genes have been described that influence the serum level of this cytokine, including SNP at position -590 (C/A). The purpose of this study was to analyze this polymorphism in a population of patient with relapsing visceral leishmaniasis. Materials and methods: The peripheral blood samples from patients with relapsing visceral leishmaniasis who had elevated IL-4 serum concentration were collected. The genomic DNA was extracted by PCR technique and primers covering promoter region for about 1200 bp long of IL-4 was amplified. Finally, the amplified DNA was examined by sequence analysis. Result: Sequence analysis of the amplified DNA showed a single nucleotide polymorphism at position -590 (C/A). Conclusion: Our results show a trend of association between the genetic combination of the A allele of -590C/A with visceral leishmaniasis. Moreover, several investigators have revealed that IL-4 is an anti-inflammatory cytokine that its serum level can influence the recovery from infections, especially from intracellular pathogens. The serum level of IL-4 results from occurring polymorphisms in promoter of this gene. Previous studies showed that the -590 (C/A) polymorphism increases IL-4 transcriptional activity, which causes the raise of concentration serum level of IL-4. The high serum level of IL-4 in patients with relapsing visceral leishmaniasis and the occurrence of SNP (C/A) at position -590 showed that this SNP could result in unfavored outcome of visceral leishmaniasis. Since this study was performed in a small population, further studies in other population are needed to confirm these results.

p-726

RECOGNITION OF TWO DIFFERENT CLADES OF SYMBIODINIUM (C90 AND D) ON PORITES COMPRESSA AND PSAMMOCORA CONTIGUA OFF KISH ISLAND (PERSIAN GULF, IRAN) BY SSCP METHOD

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Reef-building corals have unicellular dinoflagellate of the genus Symbiodinium. These symbionts provide a major part of the energy needs of the coral host through its photosynthetic activity, to the extent that some corals are dependent on the primary productivity of this symbiont for survival. Symbiodinium and their coral hosts are sensitive to environmental stresses. Molecular techniques have been used to type Symbiodinium into eight genetic clades named A, B, C, D, E, F, G and H. Iranian coral reefs are living under harsh conditions and high fluctuation in temperature in the Persian Gulf. In this survey, fragments of two species of scleractinian corals were collected on SCUBA from three reef sites off Kish

Island (Persian Gulf, Iran), the samples have been preserved by dipping in DMSO buffer, and then airbrushed with DNAB buffer. The slurry was collected and frozen at -20°C. DNA was extracted from defrosted slurries using the CTAB/chloroform method. The partial gene sequence of 28 S nuclear ribosomal (nr) of Symbiodinium (D1/D2 domains) was amplified using Symbiodinium-specific primers. Single Stranded Conformational Polymorphism (SSCP) analysis was performed and DNA sequences were determined. Using SSCP it has been identified that two coral species were found to host clade C90. Phylogenetic analyses show that Porites compressa harbors Symbiodinium clade C90 and Psammocora contigua harbor Symbiodinium clade C90 and clade D.
The presence of subclade C90 in the Iranian P. compressa and P. contigua is intriguing since C90 is the type of clade C that has been found only in East Pacific foraminiferans and hitherto, no evidence of this subclade in cnidarians.

p-727
MUTATION ANALYSIS OF CYBB GENE IN X-LINKED CHRONIC GRANULOMATOUS DISEASE IN IRAN

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Chronic Granulomatous Disease (CGD) is an inherited phagocytic disorder caused by mutations in NADPH oxidase subunits. Patients with CGD have life-threatening bacterial and fungal infections. It has been reported that most common presentation of the disease is caused by mutation in CYBB gene located on the X chromosome (Xp21.1), coding for gp91phox. Diagnosis of CGD is made by demonstrating absent or markedly reduced oxidase activity in stimulated nutrophils. In order to facilitate final diagnosis of CGD and mutation analysis of CYBB gene, we have developed molecular diagnosis of the disease. We have done CGD screening by NBT slide test, quantitative NBT and flowcytometric analysis using DHR123 for the patients and their family. Mutation screening in CYBB gene using PCR-SSCP analysis followed by sequencing, showed 7 different mutations: c. 880 C >T (Arg 290X), c.1272G>A (Trp424X), c. 676 C >T (Arg 226X) c. 271 C >T (Arg 91X), c.231C>T in (Arg 73X), c.1024G>A (Trp 337 X) and Del. AG 717/718 (fs.239 X). c.1272G>A has not been reported previously in the literature. Characterization of mutant exons is very important for detection of carriers, prenatal diagnosis and final diagnosis of disease.

p-728
THE APOAI-CIII-AIV GENE CLUSTER POLYMORPHISMS AND HYPERLIPIDEMIA

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Objectives: Genetic variations of apolipoprotein (apo) AI-CIII-AIV gene cluster and their association with hyperlipidemia have been reported in several studies. These investigations were carried out mainly in Caucasians and some studies in other races. This study was conducted to investigate the association between five polymorphic sites of the apo AI-CIII-AIV gene cluster with hyperlipidemia in hyperlipidemic Iranian subjects. These sites included XmnI site in the 5' region of the apo AI gene (C2500T substitution), MspI site in the promoter region of the apo AI gene (G75A substitution), MspI site in the first intron of the apo AI gene (C83T or G84A substitution), PstI site in the apo AI-CIII intergenic region and SstI site in the apo CIII 3' untranslated region (G3175C substitution). Methods: Total genomic DNA was prepared from the whole blood of seventy-six hyperlipidemic and seventy-five normolipidemic Iranian subjects. DNA amplification was performed by polymerase chain reaction (PCR). Genotype distribution and allelic frequencies of polymprphisms were determined and compared in hyperlipidemic and normolipidemidc subjects. The frequency of XmnI, MspI (+83bp) and SstI polymorphic sites in the hyperlipidemic subjects were significantly higher than those of the control group ($p < 0.05$). No any significant differences in frequency of the rare allelic MspI (-75 bp) and PstI sites were observed in two groups. Conclusion: Our results suggest that, the polymorphisms of XmnI, MspI (+83 bp) and SstI were associated with hyperlipidemia but polymorphisms of MspI (-75 bp) and PstI were not associated with hyperlipidemia in the selected population.

p-729
EFFECT OF APOLIPOPROTEIN E POLYMORPHISM ON SERUM URIC ACID CONCENTRATION AND LIPIDS PROFILE IN YOUNG HEALTHY SUBJECTS FROM SOUTHERN IRAN

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Background: Hyperuricaemia and dyslipidemia are risk factors for cardiovascular diseases (CVD). And apolipoprotein E (apoE) polymorphism is associated with serum lipids level, atherosclerosis and other CVDs, too. Objective: Determining distribution of apoE alleles in Southern Iran and association study of apoE gene polymorphism with serum uric acid (SUA) and lipids level as risk factors for CVD in young healthy subjects. Methods: DNA was extracted from the whole blood of 198 healthy unrelated candidates from population of Fars Province for apoE genotyping. Association of SUA level and serum lipid parameters including cholesterol, LDL, HDL, TG and cholesterol/HDL ratio with apoE gene polymorphism were studied by variance analysis. Results: The frequency of E2, E3 and E4 alleles, as three major alleles of apoE gene, were 0.063, 0.886 and 0.051, respectively. E2/E3 individuals had lower cholesterol level in comparison with E3/E3 subjects (148±37 vs. 169±33 mg/dL, P=0.026) and male E3/E4 subjects had lower SUA level in comparison with other males (3.6±0.7 vs. 5±1, P=0.015) while another parameters were not significantly different among different genotypes. Conclusion: Distribution of apoE alleles in Southern Iran is similar to Caucasians and frequency of E4 allele, as a known genetic risk factor for CVD, is the lowest reported frequency. Cholesterol

level in E2 allele-carriers was low and this means a lower risk for CVD. SUA, as a risk factor for CVD, in male E4 allele–carriers is low. The product of a genetic risk factor of a multifactorial disease might be involved to different biochemical pathways and some of these pathways might decrease the risk. This is the second report for the association between SUA level and apoE in the world.

p-730
EXPRESSION OF ROR1 AS AN ORPHAN RECEPTOR FOR DIAGNOSIS OF RENAL CANCER

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Ror1 is an orphan receptor tyrosine kinase that plays an important role in embryo development. Its expression has been traced in most organ systems during embryogenesis. The homozygous Ror1-/- mutant mice die 24 h after birth and this indicates the importance of Ror1 expression. In this study tissue and simultaneous blood samples were obtained from patients with renal cancers. Total RNA was extracted from each sample, first strand cDNA was synthesized. Ror1 gene was then amplified using specific primers by PCR strategy. Expression of Ror1 gene was measured semi quantitatively relative to the expression of a housekeeping gene, phosphoglucomutase1 (PGM1), in each sample. Our results showed that Ror1 expression in renal cancer tumor tissues was significantly higher than the cut-off value obtained from Ror1 expression in normal blood samples. Ror1 had significantly higher levels of expression in blood of patients with renal cancer as compared to that in normal blood ($P<0.001$). Age had no effect on Ror1 expression in peripheral blood and tumoral tissues of patients with renal cancers. No significant differences in Ror1 expression were observed between the different stages of renal cancers. In conclusion, we propose that detection of high levels of Ror1 expression in blood could be used as a diagnostic element and a marker for early detection of renal malignancies, provided that clinical symptoms of the disease are considered.

p-731
MUTATIONS ANALYSIS OF P53 GENE IN PATIENTS WITH HEPATOCELLULAR CARCINOMA

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Background: Hepatocellular carcinoma is the fifth cause of cancer-induced mortality throughout the world. Incidence of this disease is not identical in different parts of the world and depends on race, gender, location, diet, aflatoxin and incidence rate of hepatitis B and C. Liver carcinogenesis is a multistep process accompanied by genetic and epigenetic alterations. The p53 gene plays a major role in hepatocellular carcinoma (HCC). Our aim was to analyze the p53 mutations spectrum in patients with hepatocellular carcinoma. Method: DNA extracted from 25 formalin fixed paraffin embedded tissues of patients from hospitals in Kermanshah and Ahwaz using standard kit. Liver samples obtained from both HBV positive and HBV negative patients. To examine the prevalence of p53 mutations in HCC exon 7 and 8 of the samples were analyzed by Nested-PCR, PCR-RFLP, PCR-SSCP (single strand conformation polymorphism) and sequencing. First we analyzed exon 7 by Nested-PCR and PCR-RFLP using Hae III restriction enzyme digestion for preliminary assessment of mutation at codon 249 (R249S mutation). Then exon 7 and 8 were analyzed by Nested-PCR, PCR-SSCP and using sequencing. Results: A total of the 3 of the 25 (12%) subjects had p53 mutation. No R249S mutation in exon 7 was found in these tumor samples. SSCP analysis showed 2 mutations in exon 7 but 1 new mutation were detected in exon 8. These results were confirmed by sequencing. Conclusion: The absence of R249S mutation in exon 7 may indicate that these subjects with HCC have not been exposed to aflatoxin. A special type of p53 mutation has not been found to be associated with hepatitis B viral infection. Incidence of p53 mutations was significantly associated with the degree of differentiation of cancer. P53 mutation occurs preferentially in moderated and poorly differentiated HCC. Keyword: hepatocellular carcinoma, SSCP (single strand conformation polymorphism), P53, RFLP, R249S mutation(argenine to serine).

p-732
STATISTICAL ANALYSIS OF 7 STRS OF CHROMOSOME 21 AMONG NORTH-WEST POPULATION OF IRAN FOR DIAGNOSIS OF DOWN SYNDROME

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Microsatellites or STRs are hypervariable regions of genome with repeated units up to 13 bp. The high rate of length polymorphism of STRs arise from variations in the number of tandem repeated units resulting in production of allele length variation in different people. Through the process of PCR, different STR markers can be amplified and the number of alleles that they produce can be scored. The aim of this study was sellecting and applying the polymorphic microsatellite markers for diagnosis of Down syndrome patients and determining the parental origin of extra chromosome 21 in this group of patients. The percentage of polymorphism in microsatellites located on chromosome 21 among the population of Northwest was going to be studied. DNA samples of 50 people from population of Northwest were collected randomly. Using 7 STRs of chromosome 21 including: D21S11, D21S1411, D21S1910, D21S215, D21S192, D21S17 and D21S1270, these samples were studied by applying SSR-PCR. The resulting alleles were analyzed by Power marker and PopGene software. Then 30 Down syndrome newborns diagnosed by clinical manifestation were

studied by this technique. High rate of polymorphism in the mentioned population were observed on D21S11, D21S1411, D21S1910 respectively. The frequency of heterozygosity of these 3 markers was as follows: D21S11: 66%. D21S1411: 80%. D21S1910: 82%. We could diagnose most of the affected newborns with Down syndrome by SSR-PCR using 3 highly informative markers: D21S11, D21S1411 and D21S1910. In addition this technique is used for prenatal screening of high-risk pregnancies.

p-733
CHARACTERISATION OF RECOMBINANT HEPHAESTIN

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Iron is an essential nutrient for systemic iron hemeostasis. Dietary iron enters the intestinal epithelium via the brush border reductase/transporter Dcytb /DMT1 or haem pathway and exists through the basolateral membranes. The basolateral transfer of iron requires two components: a membrane transport protein Ireg1 and ceruloplasmin (Cp) homologue known as hephaestin (Hp/Heph), which was identified as the defective gene in the sla (sex-linked anaemia) mice and plays a critical role in intestinal iron absorption, ascribed to its multicopper ferroxidase (Fe 2+ to Fe3+) activity. Knowledge of the synthesis, distribution, activity and regulation of hephaestin will help to understand its role in cellular iron efflux. The aim of this investigation was to investigate the role of bioactive recombinant hephaestin in different cell lines e.g. COS, CHO, MDCK, Caco2 and HepG2 and assess its ferroxidase activity, and its ability to interact with apo-transferrin and Ireg1. The construction of Hpsec-GFP and its expression is described which demonstrates a strong perinuclear signal with punctate signals in the cytoplasm, gradually decreasing in intensity, moving from the nucleus to the plasma membrane. Localisation data for recombinant GFP-tagged full-length hephaestin showed similar patterns of expression in different cell lines. In cell lines tested hephaestin was strongly localised to the perinuclear and cytoplasmic regions. Localization of wild-type hephaestin to the basolateral membrane is in agreement with its anticipated function as a ferroxidase facilitating iron efflux. However, the precise subcellular localisation of hephaestin in enterocyte and the site of interaction with other iron homeostasis proteins are not clear.

p-734
PPAR GAMMA TRANSCRIPTION ACTIVITY AND TELOMERASE REGULATION IN BREAST CANCER

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Breast cancer is the most common malignancy affecting North American women. In over 90% of breast carcinomas, telomerase is upregulated. Telomerase is a ribonucleoprotein consisting of a protein catalytic subunit (hTERT) and a RNA subunit (hTR). hTERT has been shown to be the rate limiting subunit of telomerase and is found in 85-90% of all cancer cells. Telomerase expression confers limitless replication to a cancer cell. Inhibition of telomerase activity by promoting the differentiation of cancer cells could be used to treat breast cancer. Several studies suggest an important role for peroxisome proliferator-activated receptor γ (PPARγ) in induction of terminal differentiation as a potential therapeutic approach to certain human malignancies. Upon activation by its ligands, PPARγ heterodimerizes with the retinoid X receptor (RXR) and binds to a recognition sequence, DR-1, in the promoter regions of their target genes. Interestingly, RXR activation together with retinoic acid receptor (RAR) binding inhibits telomerase activity and cell growth in breast cancer cell lines. Given its involvement in tumorgenesis, we hypothesize that PPARγ is a potential candidate for the regulation of telomerase in breast cancer. To find a model for our study, three different breast cancer cell lines (MCF7, MDA-MB 231, and T47D) were tested for the expression of PPARγ and hTERT. We found that MDA-MB 231 expresses both PPARγ and hTERT, whereas MCF7 mainly expresses hTERT and T47 expresses only PPARγ. Based on these findings the MDA-MB 231 cell line was selected as an in vitro model. These cells were treated with different concentrations of troglitazone (a ligand for PPARγ) at various times. Our results show that troglitazone arrests cell proliferation and holds cells in G0-G1 phase. We also observed that cell cycle arrest in troglitazone treated cells is associated with reduced telomerase activity (TRAP assay). Real-time PCR for hTERT in treated cells showed that troglitazone suppresses telomerase at the transcriptional level. Using BADGE, a PPARγ antagonist, we could prevent the effects of troglitazone on telomerase activity. Furthermore, our preliminary data showed that troglitazone does not cause a reduction in telomerase activity in other cell lines (MCF7 cells and T47 cells). In summary, this study showed that troglitazone could reduce telomerase activity and mRNA transcript levels in a breast cancer cell type expressing both PPARγ and hTERT, and therefore suggesting that these effects are PPARγ dependent.

p-735
MOLECULAR DIAGNOSIS OF DIGEORGE SYNDROME 3 MB COMMON DELETION BY SEMI QUANTITATIVE PCR AND ITS COMPARISON WITH FISH

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Background: DiGeorge syndrome is the most common microdeletion syndrome with an estimated incidence of 1/4000 live births. The majority of patients (90%) share a common 3 Mb hemizygous deletion of 22q11.2 (Typically Deleted Region, TDR). The remaining patients include those who have smaller deletions nested within 3 Mb (8%) and a few with rare deletion that have no overlap with the TDR. These deletions result from unequal recombination at meiosis

between chromosome-22 specific low-copy repeats (LCR22s). FISH is the standard technique to detect the common microdeletion but it has shown some drawbacks. Because of using expensive probes it is not cost effective and cannot detect smaller microdeletions. Methods: We designed a novel semi quantitative PCR based technique, which is able to detect small microdeletions, based on known intra and extra-TDR's STRs and markers. We developed a semi quantitative PCR to detect copy number of the interest region. Results: The PCR product was in log phase between 22 and 28 cycles. We then tested our semi quantitative method on known FISH positive DiGeorge cases. This novel method was able to detect common microdeletions. Application of our semi quantitative PCR on patients with congenital cardiac anomaly of conotruncal type detected one patient with a microdeletion of TDR. This result was confirmed by FISH. Conclusion: Our novel semi quantitative PCR method was able to detect the common 3MB microdeletion of DiGeorge syndrome. However further study is needed to find the exact sensitivity. If proved to be more sensitive especially in detecting smaller microdeletions, this method can replace FISH in less equipped laboratories or when a small microdeletion is suspected.

p-736
MOLECULAR PHYLOGENETICS OF THE FONSECAEA SPECIES BASED ON ITS rDNA DATA SETS

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Fonsecaea, especially Fonsecaea pedrosoi is the most common etiologic agent of human chromoblastomycosis. Chromoblastomycosis is a cutaneous and subcutaneous infection characterized by verrucose lesions and a tissue phase that consists of muriform cells. The disease is found world wide, but most reports are from tropical and subtropical climates. A phylogenetic study was performed in 55 strains morphologically identified as Fonsecaea species from clinical specimens and living environments in central and South America based on sequence analysis of the ribosomal internal transcribed spacer 1 (ITS), 5.8S and ITS2 region. The samples were grown in PDA (potato dextrose agar) and MEA (Malt extract agar) medium for 15 days at 25^{o} C for DNA extraction. DNA was extracted with ultra clean microbial DNA isolation kit. Then PCR was performed for all the samples with specific primers (V9G and LS266), amplified rDNA fragments were subjected to electrophoresis in 1% agarose immersed in Tris-borate ethylenediamine tetraacetic acid buffer. The PCR product was purified using the GFX-columns Kit (pharmacia). And the ITS region were sequenced directly from amplicons using the primers pairs ITS1 and ITS4. The Strains of Fonsecaea were divided into two major clades: Group A, representing Fonsecaea pedrosoi and Group B, representing Fonsecaea monophora. We identified new lineages: Group B was further clustered into two subgroups B-1 and B-2, which were phylogenetically distinct from each other.

O-737
RELIABILITY OF THE C-TERMINUS PART OF PLASMODIUM FALCIPARUM AND P. VIVAX MEROZOITE SURFACE PROTEIN-1 AS A COMPONENT OF MULTI-STAGE, MULTI-COMPONENT REGIONAL MALARIA VACCINE IN IRAN

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C-terminal region of Merozoite Surface Protein 1 of Plasmodium falciparum (PfMSP-1) and P. vivax (PvMSP-1) are main candidate vaccine antigens. However, sequence heterogeneity of this region is the main factors that impediment of its efficacy in vaccine development. In this study we analyzed the sequence variation of the C-terminal region of merozoite surface protein-1 of P. falciparum (Pfmsp-119) and P. vivax (Pvmsp-119) genes as the most promising blood stage vaccine target antigens, in 80 P. vivax and 92 P. falciparum infected blood samples collected from areas with different malaria endemicity in Iran. The presence of polymorphism in this region may compromise its use as a vaccine candidate. All P. vivax samples have shown 100% conserved sequences among northern and southern isolates, however the MAD20 allele was found significantly among P. falciparum clinical isolates in south (88.2%). Furthermore, 7.6% of blood samples were K1 and 4.2 % contained both K1 and MAD20 allelic types. MAD20 allelic type showed 4 different allelic forms due to variations in 5 positions: 1644(E/Q), 1691(T/K), 1700(S/N), 1701(R/G) and 1716(L/F), while the K1 allelic type showed no polymorphism. These results are discussed with regards to evaluation of these vaccine antigens in both malaria species, and in comparison with the studies that were conducted in other areas in Southeast Asia, and Africa.

p-738
ASSOCIATION OF GENETIC MUTATIONS IN CHLOROQUINE RESISTANCE TRANSPORTER (CRT) WITH RESISTANCE TO CHLOROQUINE IN CLINICAL PLASMODIUM FALCIPARUM IN IRAN

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We assessed the prevalence of polymorphisms in the Plasmodium falciparum chloroquine resistance transporter (pfcrt) and Multi Drug Resistance-1 (pfmdr1) genes before (n=158) and after treatment (n=25) with chloroquine in Iranian P. falciparum strains. Nested PCR and PCR/RFLP methods were used to detect SNPs in pfcrt gene at positions K76T, A220S, Q271E, N326S, I356T, R371I and in pfmdr1 gene at positions N86Y, Y184F, S1034C, N1042D, D1246Y. Only 76T (97%), 220S (97%) and 326S (97%) for pfcrt gene in pre treatment samples and in pfmdr1 86Y (34.8%), 184F (7.6%) mutant allele was detected, with no mutation at other positions. To test the hypothesis that in vivo selection of mutant pfcrt alleles occurs after chloroquine treatment, pfcrt and pfmdr1 polymorphisms were compared among 25 post treatment paired samples from patients' parasitologic failure. Analysis of the post treatment samples showed that high levels of CQ pressure caused strong sellection of pfcrt76T (100%),

220 S (96%) and 326 S (96%) polymorphisms in Iran. The frequency of the mutant pfmdr1 86Y allele was 40% among post treatment isolates and all also carried the mutant pfcrt 76T allele. In conclusion, these data point to high frequency of drug-resistance mutations in P. falciparum in southeastern Iran, and strongly support that CQ as the first line drug for treatment of falciparum malaria in Iran, is an inadequate drug for this region.

p-739
IL-1, IL-1R, IL6 AND TNFα GENE POLYMORPHISMS IN IRANIAN PATIENTS WITH MULTIPLE SCLEROSIS

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Cytokine gene polymorphisms have been extensively studied in association with different diseases. The role of cytokines gene polymorphisms in multiple sclerosis (MS), as a chronic Immune-mediated neurodegenerative disease has been previously reported in the other countries. Materials and Methods: In this investigation for determining pro-inflammatory cytokine gene polymorphisms 100 RRMS Iranian patients and 140 healthy individuals as control group were selected. DNA of the samples was extracted by a modified salting out method. Cytokine gene single nucleotide polymorphisms including TNF-α (-308 G/A and -238G/A), IL-1α (-889 C/T), IL-1□(-511C/T and +3962C/T), IL-1R (pst1 1970C/T), IL-1RA mspal (11100C/T), IL-6 (-174 G/C and nt 565 G/C) were determined using the PCR-SSP method. Allele and genotype frequencies for both groups were determined using statistical software chi square, odds ratio and p value. The results of our data indicated that the frequency of IL-1α TC genotype (66% vs. 45.6%, p = 0.002), IL-1β + 3962 TC genotype (61 % vs. 41.4%, p = 0.004), IL-1R T allele (56% vs. 37.9% p = 0.0001), IL-1 RA Mspal 11100 TC genotype (58% vs. 40%, p = 0.009), IL-6 -174 C allele (56% vs. 36.3%, p = .00003) and CG genotype (89% vs. 66.9, p = 0.0001), TNF-α -308 A allele (29% vs. 14%, p = 0.0002), AG genotype (57.6% vs. 28.5%, p = 0.00001) increased significantly in the patients versus healthy people. On the other hand the frequency of IL-1α TT genotype (1% vs. 8.8% p = 0.028), IL-1R C allele (44% vs. 62.1, p = 0.0001) and CC genotype (14% vs. 38.6%, p = 0.00006), IL-1 RA Mspal 11100 TT genotype (40% vs. 57.1, p = 0.013), IL-6 -174 G allele (44% vs. 63.7, p = 0.00003), GG genotype (0% vs. 30.2%, p = 0.00) and TNF-α -308 G allele (71% vs. 85.8, p = 0.0002), GG genotype (42% vs. 71.5%, p = 0.000001) decreased significantly in the patients versus healthy people. Conclusion: It is suggestive that alleles and genotypes of pro-inflammatory cytokines have a profound role in the pathogenesis of multiple sclerosis.

p-740
QUANTITATIVE ANALYSIS OF SMN1 GENE DELETION BY REAL-TIME PCR

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Spinal Mascular Atrophy (SMA) is an autosomal recessive neuromuscular disorder caused by mutation in the SMN1 (survival motor neuron) gene, mainly intragenic deletion, the most common of which are deletion in exons 7 and 8. The disorder is subdivided into three clinical groups (type I-III) on the basis of age of onset and clinical severity. The molecular basis for variation in clinical manifestation depends on the copy number of SMN2 gene in each patient. Until now, several densitometric based semi quantitative PCR methods for SMN1 analysis have been developed for carrier detection. However, these methods were not accurate and easily submitted to errors. Recently reported quantitative real-time PCR assays allow the specific amplification of only SMN1 gene, which avoid these problems. However, these tests are probe-based (TaqMan) which makes them very expensive. Considering these problems, we utilized a reliable quantitative real-time PCR method using SMN1 gene specific primers and SYBR Green I dye for the carrier detection of SMA. The comparative Ct (threshold cycle) method was used to quantify the copy number of SMN1 gene. This test allowed us to analyze a large number of samples efficiently. Here, we report the results of quantitative analysis of SMN1 gene in SMA patients and suspected carriers.

p-741
DETECTION OF DYSTROPHIN GENE DELETIONS IN FEMALE DMD CARRIERS BY QUANTITATIVE REAL-TIME PCR

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Duchenne muscular dystrophy (DMD) is a lethal X-linked recessive disorder affecting 1 in 3500 male births. DMD is caused by mutations in the dystrophin gene located at Xp21.2, which encodes protein of the membrane cytoskeleton in skeletal muscle (dystrophin). The dystrophin gene with the size of 2.4 Mb and containing 79 exons that encode 14 kb mRNA is the largest encoding gene found in nature to date. This probably accounts for the high frequency and high proportion of de-novo mutations in this gene. Two-thirds of DMD mutations are deletions. Detection of exonal deletions in male hemizygous affected individuals is currently carried out by multiplex qualitative PCR. However, analysis of such deletions in female carriers could not be done due to the fact they are homozygous for X chromosome. To overcome this problem various techniques have been adopted so far including QF-PCR, FISH and linkage analysis utilizing STR and RFLP markers within dystrophin gene. Nevertheless, these methods are labour intensive and time consuming. Here we present a quantitative real-time PCR based on SYBR Green I technology detecting deletions within the dystrophin gene in female carriers. Our method is based on comparative quantification using conventional duplex PCR, Real-time PCR and gender determination. Real-time PCR was specifically developed to quantify specific DNA targets through the monitoring of product formation. This technology has been successfully applied for the detection of hemizygous deletions in different genetic disorders. The comparative Ct (threshold

cycle) method was used to quantify the copy number of dystrophin gene. We report the results of quantitative analysis of dystrophin gene in DMD patients and suspected carriers.

p-742
A NOVEL MUTATION IN SLC19A2 GENE IN AN IRANIAN PATIENT WITH THIAMINE RESPONSIVE MEGALOBLASTIC ANEMIA SYNDROME

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Thiamine – Responsive Megaloblatic Anemia (TRMA) or Roger syndrome is a rare autosomal recessive disorder with childhood onset. This disorder is characterized by the occurrence of multiple clinical manifestations including megaloblastic anemia, diabetes mellitus and sensorineural deafness, responding in varying degrees to thiamine treatment. The gene SLC19A2 that codes for a thiamine transporter is responsible for this syndrome and it is located at chromosome 1q. To date 15 different mutations have been reported in 28 families worldwide. Here we present a new case with a novel mutation. A 20 years old male patient with characteristic features of TRMA with good response to thiamine therapy was identified. His SLC19A2 gene was screened by direct sequencing and a single nucleotide base substitution in homozygous form was found. This mutation leads to a premature stop codon (W233X), and can be considered diseases causing because of its nature. Considering the limited number of affected cases reported so far in the literature, it is evident that TRMA is a very rare condition with heterogenous molecular basis. As far as its distribution is concerned, out of 28 reported TRMA families worldwide, five families including this present case are from Iran who had different mutations. The rest of the patients were from Indian subcontinent (India, Kashmir and Pakistan) suggestive of higher frequency in Asian populations.

p-743
COMPARATIVE MOLECULAR STUDIES ON ANOPHELES SPECIES FROM FARS PROVINCE, IRAN

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Due to ecological differences, malaria as a disease behaves in different ways even in areas with comparable climates. In past, almost all provinces of the Iran were highly endemic for malaria. However, malaria is now restricted to the southeastern part of country with indigenous and imported cases. Among the other parts of the country, Fars province is a malaria prawn region, with a quickly changing pattern of ecology and zoogeography. In current study, anopheles mosquito specimens were collected from seven districts in Fars province including; Estahban, Sepidan, Neiriz, Mehr, Farashband, Lamerd and Arsanjan during July-September 2006. Morphological identification carried out by using the key to Iranian Anophelines. Results showed that six species of Anophlelines are prevalent in these regions, including Anopheles stephensi, An. superpictus, An. dthali, An. maculipennis, An. sergenti and An. multicolor. In order to verify the presence of An. maculipennis species complex in Fars province, further molecular studies carried out by using rDNA-ITS2 sequencing. Three sequenced specimens have shown 100% similarity with An. maculipennis s.s. rDNA sequence deposited in GenBank. This is the first report on molecular identification of maculipennis complex from Estahban and Sepidan districts in Fars province. Presence of four main malaria vectors in this province is an indication for continuation of surveillance, especially vector control programs.

p-744
MOLECULAR IDENTIFICATION OF A NEW MEMBER IN ANOPHELES HYRCANUS GROUP

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Members of Anopheles (Anopheles) hyrcanus Group of mosquitoes are important malaria vectors, almost impossible to differentiate them morphologically in the adult and larval stages. In the current study the rDNA-ITS2 sequences were identified in 25 specimens of Anopheles hyrcanus collected from Ardebil, Guilan and Khuzestan provinces in Iran. The total size of amplified fragment was ~600bp. The length of ITS2 ranged from 436 to 447bp, with the GC content of 45.19 to 46.79%, comprising the presence of two species within this complex in Iran. Phylogenetic analysis based on ITS2 revealed that different populations of An. hyrcanus group are clustering in two main branches, which probably could be considered as two species within this group. Anopheles stephensi seems to be the closest taxa to An. hyrcanus among the three species that have been used as out groups. Nested polymerase chain reaction (PCR) assay for the detection of malaria parasite species showed that none of the studied individuals were positive for Plasmodium spp. The outcoming results introduced a more reliable molecular technique for identification of inter and intra-species genetic variation in this species group, and provided preliminary evidence on the status of vectorial capacity in An. hyrcanus.

p-745
IDENTIFICATION AND COMPARATIVE SEQUENCE ANALYSIS ON CYP6Z1 AND GSTE2 GENES IN ANOPHELES STEPHENSI POPULATION FROM IRAN AND PAKISTAN

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Anopheles stephensi is the main malaria vector in Iran and eastern Mediterranean region. Although the use of insecticides can reduce the vectors population, many vectors show

resistance to insecticides due to variety of genetic changes. Glutathione-S-transferses and Cytochrome P450s are two important superfamily genes involved in metabolic insecticide resistance. Anopheles stephensi specimens were collected from Sarbaz, Khash and Nikshahr districts in Sistan and Baluchistan province of Iran and Karachi in Pakistan. cyp6z1 and gste2 genes cloned and sequenced using primers designed based Anopheles gambiae genome. Primers amplified 1258 bp fragment in cyp6z1 and 489-492 bp in gste2 genes. Sequence of cyp6z1 in Anopheles stephensi without any introns, had 79% similarity with the Anopheles gambiae cyp6z1 gene in nucleotide and 86% in amino acid sequences. gste2 sequence of An. Stephensi including part of exon II, exon III, and complet sequence of intron II. There was not intraspecific variation in Iranian strains. Sequences of Pakistani samples showed 100% identity with Iranian strains. Kazeroon strain (as a susceptible strain) showed 99% identity with both Pakistani and Iranian strains. It had transversion and transition in 105th (C/G) and in 174th (A/C) nocleotid. Pakistani and Saravan strains had transition (A/G, C/T) in 243th and 351th nucleotide. Beside these exchanges, in amino acid sequences all strains were 100% identical. These data may be useful for implementation and evaluation of malaria control programs, population genetics and molecular resistance in border area between Iran and Pakistan.

p-746

MOLECULAR AND STRUCTURAL ANALYSIS OF LECTIN AND LECTIN-RELATED GENE IN IRANIAN MAIN MALARIA VECTOR ANOPHELES STEPHENSI

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Malaria is one of the major vector-borne infectious diseases. Malaria parasites are transmitted by Anopheles vectors. Interactions between parasites and vector gut wall may be mediated by the carbohydrates on the surface of parasites and lectin such as fibrinogen-related proteins in the vector gut. In current study we used primers designed based on fibrinogen gene in Anopheles gambiae. Anopheles Stephensi specimens were collected from Sistan and Baluchistan Province of Iran and DNA was extracted using Collins method. A 380 bp fragment was amplified by these primers (FBN9) with 56.84% GC content. These had 99% similarity within Iranian specimens and had 90% similarity with Anopheles gambiae FBN9 gene. Their amino acid sequences had 92% similarity with Anopheles gambiae. In order to structurally identify the lectin gene in An. stephensi, primers were designed based on Lectin mRNA in Anopheles gambiae. Anopheles stephensi specimens were collected from Khash, Sarbaz, Iranshahr, Nikshahr, Chabahar districts in Sistan and Baluchistan province. Cloning, followed by sequencing identified an 897bp fragment in all those strains of An. stephensi which showed 99% similarity in intra-species level. However, this fragment has no similarity with the available sequences in GenBank. It is necessary to do further research to obtain more information about the structure of lectin and lectin related genes in Anopheles vectors to study the role of these genes in interaction between plasmodium and Anopheles to find candidate molecule for transmission blocking vaccine.

p-747

EXPRESSION AND REGULATION OF KLOTHO AND OTHER CRITICAL PROTEIN MOLECULES AS THE REGULATORS OF CALCIUM HOMEOSTASIS IN THE KIDNEY OF AROMATASE DEFICIENT MICE (ARKO)

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Klotho was initially identified in mice as a gene associated with suppression of several ageing phenotypes including short life span, infertility, ateriosclerosis, skin atrophy, osteoporosis and abnormal calcium reabsorption. The klotho gene encodes two forms of protein, a type I single transmembrane 130 kd glycoprotein with α-glycosidase activity, predominantly expressed in tissues involved in calcium homeostasis, such as kidney, parathyroid glands and choroids plexus in the brain. The second type of klotho expressed as splice variant is without the second α-glycosidase like repeat. A near full length form of Klotho protein lacking the transmembrane domain and cytoplasmic fragment is secreted into the blood and cerebrospinal fluid. Recent studies have shown that in the distal convoluting tubule of kidney (DCT), the Klotho protein co-localizes at the subcellular level with epithelial calcium channel protein (TRPV5), Na+/Ca2+ exchanger (NCX1) and the cytosolic calcium binding protein calbindin-D28k. Up-regulation of Klotho and TRPV5 by vitamin-D in kidney has also been reported and it has shown that mice with a disrupted klotho gene have abnormal calcium and vitamin-D metabolism, and develop hyperphosphatemia and vascular calcification. The DCT plays a major role in maintenance of total body calcium homeostasis controlled by parathyroid hormone (PTH), vitamin D3 and other steroids including estrogen. Aromatase is the only known enzyme that synthesizes estrogens and thus aromatase deficient mice provide a convenient model to study the effects of estrogen deficiency. In a recent study we showed that the expression of these calcium-transporting proteins (TRPV5, calbindin-D28k, NCX1 and PMCA1) in kidney of ArKO mice was down regulated compared to wild type (WT). With the exception of NCX1 the decrease was reversed in estrogen treated ArKO female mice. Colocalization of Klotho with these proteins in kidney, suggests that klotho may be involved in regulation of renal calcium reabsorption and homeostasis. In present study, we used both in vitro (MDCK and DCT kidney cell lines) and in vivo models (WT and ArKO mice) to investigate the expression of klotho at the transcriptional and protein levels by real time PCR and immunoblotting methods. Our results showed a significant downregulation of klotho both at mRNA and protein levels in response to estrogen. Based on our results, estrogen regulates the expression of calcium transporting proteins in kidney and it may also indirectly controls the expression of klotho in kidney. This might contribute in further understanding of calcium homeostasis in the absence of estrogen.

p-748

ASSOCIATION OF THE HUMAN GLYCOPROTEIN PC-1/ENPP1 GENE WITH RISK OF TYPE 2 DIABETES

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The ENPP1/PC-1 gene inhibits insulin signal transduction pathways. Previous studies in different ethnic populations have demonstrated that the K121Q variant of the ENPP1 gene have a significant functional role in determining susceptibility to Type 2 diabetes (T2D). The possibility of other ENPP1 variants influencing these phenotypes has received little attention. To assess whether the K121Q or other variants in ENPP1 and haplotype block in linkage disequilibrium (LD) including K121Q has any impact on Type 2 diabetes in Japanese, we undertook a large-scale population-based association study using 911 T2D patients and 876 controls. Single locus association test in K121Q variants, showed no significant differences in either genotype distribution ($p = 0.95$) or allele frequency ($p = 0.83$) between T2D and control groups. However a polymorphism in intron 20 of ENPP1 gene showed a strong contribution with T2D in codominant and recessive model ($p = 0.0002$ and 0.003, respectively). Based on haplotype association analysis, no difference was observed in the haplotype frequency between patients and controls. Interestingly, the Q121 allele frequency was showed a marked ethnic variation (77.3% in African- Americans, 16.7% in European Americans, 10.5% in Japanese and 4.2% in Han Chinese). Consequently, the pair-wise FST value (a classic measure of genetic distance) showed highly significant differentiations between African- American and non- African-American populations (FST>0.3). We concluded that a new polymorphism other than K121Q has a key role for risk of type 2 diabetes in Japanese. However, the K121Q may play a role in the inter-ethnic variability in insulin resistance and T2D.

p-749

IDENTIFICATION OF THE FUNCTION OF RRP47 PROTEIN, THE COFACTOR OF THE EXOSOME COMPLEX

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Rrp47 protein, the homologue of human C1D, is a nonessential yeast protein that functions as a substrate-specific nuclear cofactor for exosome activity, the 3' end processing of stable RNAs including ribosomal RNA. Rrp47p is also a nucleic acid binding protein that shows no sequence homology with any other known DNA or RNA binding proteins. This suggests that the protein may contain a unique feature which facilitates the nucleic acid binding. There is no structure available for the protein so far. However the C terminus of the protein is predicted to be highly unstructured with a tail of lysine residues, which may contribute, to the nucleic acid binding. To determine the role of the C terminus of the protein in binding to DNA in vitro a mutant strain for the expression of the C terminal deletion of the Rrp47p was constructed. The DNA binding affinity of the mutant was then determined in vitro by an electrophoretic mobility shift assay (EMSA). In this study I have also analysed the ribosomal RNA processing function of the Rrp47p without a C terminal tail in vivo. This has been carried out by construction of a second mutant for expression of the C terminally truncated protein in yeast. I have also conducted the chemical cross linking of the Rrp47p to determine the structural organisation of the protein without a C terminal tail. In an EMSA assay with a C terminally truncated Rrp47p, the DNA binding affinity of the truncated protein was shown to be less than the full length protein in vitro. This result clearly indicates that the lysine tail of the protein C terminus stimulates the binding of the Rrp47p to DNA. Moreover, the data from chemical cross-linking assay demonstrated the formation of higher order complexes (tetrameric complexes) by Rrp47p. In this assay, the Rrp47p was also shown to be still a tetramer without a C terminal tail. These observations thus indicate that the N terminus of the protein is sufficient for protein- protein interaction. Furthermore the ribosomal RNA analysis of the Rrp47p without a C terminal tail in vivo indicated that the expression of the truncated Rrp47p complements the slow growth phenotype of rrp47- Δ mutant. This finding is also consistent with the growth rate analysis of the truncated Rrp47p as the expression of the protein without the C terminal tail also complements the slow growth phenotype of rrp47- Δ mutant. These results therefore demonstrate the requirement of the C terminal tail of the Rrp47p to stimulate the nucleic acid binding both in vitro and in vivo.

p-750

METHYLENTETRAHYDROFOLATE REDUCTASE C677T GENOTYPE AFFECTS PROMOTER METHYLATION OF TUMOR-SPECIFIC GENES IN SPORADIC COLORECTAL CANCER THROUGH AN INTERACTION WITH FOLATE/VITAMIN B_{12} STATUS

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Inappropriate epigeneticprograming has been associated with human cancer. Methylentetrahydrofolate reductase is a key enzyme that regulates entry of folate into the methylation cycle. We examined the associations between *MTHFR C677T* genotype, and promoter methylation of *P16*, *hMLH1*, and *hMSH2* tumor-associated genes among 151 Iranian cancer patients. Eighty-six patients from whom fresh tumor samples were obtained and 81 controls were also examined for serum folate and vitamin B_{12} concentrations. 29.1 % of cases had tumors with at least one methylated gene promoter. Compared with controls, the *CT* and *CT/TT* genotypes showed an increased risk of tumor methyaltion OR = 2.4 (95% CI 1.1 – 5.3), and OR = 2.0 (95% CI 1.03 – 4.1), respectively. We observed an increased risk of tumor methylation when serum folate/vitamin B_{12} levels are high. Adjusted odds ratio for high (above median) versus low (below median) serum folate/vitamin B_{12} levels were 4.2 (95% CI 1.23 – 14.2), and 3.4 (95% CI 1.1 – 10.5), respectively. In contrast to the *CC* genotype, *C677T* genotypes showed significant associations with high serum folate/vitamin B_{12} levels for tumors methylation. We conclude that high concentrations of serum folate/vitamine B_{12} are directly associated with the risk of promoter methylation in tumor-specific genes, and this relationship is modified by *MTHFR C677T* genotypes.

Nanobiology

p-751
NANOPARTICLES-MEDIATED WILD-TYPE P53 GENE DELIVERY

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The development of vectors that are capable of efficient gene delivery is crucial to the success of gene therapy. Gene transfer using nonviral vectors have been developed and are devoid of immunogenicity. Nonviral vectors can also be proposed for targeted cell interaction. Among nonviral vectors biodegradable nanoparticles have been widely studied and found effective in vitro and in vivo and devoid of toxicity whatever the administration route. We have developed non-viral vectors with the goal of correcting genetic abnormalities in cancer cells. Biodegradable nanoparticles formulated using a biocompatible polymer has the potential for a sustained gene delivery. Our hypothesis is that nanoparticles-mediated gene delivery would result in sustained gene expression, and hence better efficacy with a therapeutic gene. Nanoparticles loaded with wild type-p53 DNA demonstrated greater and sustained antiproliferative activity in vitro as compared to that with naked DNA and DNA-liposome complex. The greater efficacy of wt-p53 DNA-loaded nanoparticles was attributed to sustained intracellular DNA delivery and gene expression. The mechanism of inhibition of tumor growth with wt-p53 DNA-loaded nanoparticles was related to higher apoptosis of tumor cells than controls and the induction of antiangiogenic protein, thrombospondin-I that inhibited tumor angiogenesis. The aim of the proposed studies was to determine the efficacy of p53 gene-loaded nanoparticles in inducing antiproliferative activity in a cancer cell line. These vectors provide improved methods with which to treat cancer in the clinical setting with gene therapy.

p-752
THE REMOVAL OF PHENOLIC COMPOUNDS FROM WASTEWATER BY ENZYMIC PROCESSE

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Many industries generate phenolic pollutants during their manufacturing processes. Most of these compounds are toxice and have been classified as hazardous pollutants. Soybean peroxidase (SBP) catalyzes the oxidation and polymerization of phenolic compounds in the presence of hydrogen peroxide. The polymerized products can easily precipitate and be filtered from solution. Experiments were conducted with SBP for investigating the effective parametersfor the removal of phenolic compounds, including phenol, o-cresol and m-cresol, from synthetic wastewater. The results showed that, an increase in hydrogen peroxide up to the optimum [H2O2] / [substrate] ratio leads to an elevated removal of phenolic compounds. Higher cocentrations of H2O2 inhibited the reaction. Treatment in the presence of PEG (polyethylen glycol) as an additive increased the effect of the enzyme. A very low amount of PEG can significantly reduce the enzyme needed. Application of different cocentrations of the enzyme (0.5-4 u/mL based on Guaiacol assay) in the reaction mixture showed a positive regression between enzyme cocentration and phenols removal. Studies to determine the optimum pH for enzyme activity revealed that elimination of phenols was improved in neutral pH. In this study the effect of soybean seed-hulls on the elimination of phenolic compounds in synthetic wastewater was also demonstrated. After 2 hours, with an increase in soybean seed-hulls (10-80 g/lit), the removal of phenolic compounds was improved, but after 24 hours no significant difference was observed between increase in the concentration of seed-hulls and phenol removal.

O-753
QUANTUM DOT BASED DNA NANOSENSOR FOR THE DETECTION OF CLOSTRIDIUM BOTULINUM TYPE A, B AND E IN SELECTED BIOLOGICAL SAMPLES

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Rapid and highly sensitive detection of *Clostridium botulinum* in biological samples is critical because of its extra toxicity. This paper introduce a new ultrasensitive nanosensor based on fluorescence resonance energy transfer (FRET) capable of detection low concentration of a DNA fragment from *Clostridium botulinum* types A, B and E. We first predicted conserved sequences of genes encoding the botulinum toxin types A, B and E in 61 strains using gene banks data. Then the proper suitable ssDNA probes for capture and reporter of each conserved sequence and the whole system were designed. This system uses quantum dots (QDs) linked to DNA probes to capture DNA targets. The target strand binds to a dye-labeled reporter strand thus forming a FRET donor-acceptor ensemble. The QD also functions as a concentrator that amplifies the target signal by confining several targets in a nanoscale domain. Unbound nanosensors produce near-zero background fluorescence, but on binding to even a small amount of target DNA they generate a very distinct FRET signal.

p-754
INDUCTION OF AMYLOID FIBRIL FORMATION BY ALPHA-CHYMOTRYPSIN BY TWO DIFFERENT METHODS

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Amyloid or amyloid-like fibrils are elongated, unbranched protein fibrils with diameters ranging from 5 to 14 nm. Several lines of evidence suggest that amyloid fibril formation is a

generic property of polypeptide chains. In the present study, we have employed two different methods to drive α-chymotrypsin, a well-known serine protease with all-β fold, toward amyloid fibril formation. In method I, a combination of the acidic pH and high temperature (above Tm of protein denaturation) was used. In method II, a low concentration of the fluorinated alcohol trifluoroethanol was employed instead of high temperature. The possible amyloid fibrils formation was investigated through dye (Congo red, thioflavin T) binding assays, far-UV circular dichroism spectroscopy and transmission electron microscopy images. It was demonstrated that, after 48 hours, alpha-chymotrypsin forms fibrils with diameters of about 5 nm in method I and 10 nm in method II. The initial conformation of protein induced by method I seemed to be completely unfolded, but in the case of the method II, it appeared to be a molten-globule like species. Taking the results of this study together, it can be concluded that diverse conformations of alpha-chymotrypsin induced under various circumstances are prone to amyloid aggregation and the morphology of aggregates is subtly controlled by the environmental conditions.

p-755
INHIBITION OF EGFR BY ANTISENSE USING DENRIMERIC GENE DELIVERY STRUCTURES IN MCF7 CELLS

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Breast cancer appears to be the second most lethal type of cancer which indeed necessitates implementation of novel therapeutics to target the involved oncogenes. The human epidermal growth factor receptor (EGFR) is an oncogene overexpressed in solid tumors (e.g., 82-90% overexpression in breast cancer) and results in tumor growth and development. Thus, inhibition/suppression of this gene at transcription level may control the signaling pathways resulting in tumor inhibition. In this study, to pursue this hypothesis, we aimed to investigate the expression of EGFR in breast cancer MCF-7 cells upon treatment with anti-EGFR antisense oligdeoxyonucleotides (ODNs). Briefly, cells were cultured at a seeding density of 4 $\times 10^4$ cells/ cm2 in MEM supplemented with 1% NEAA, 10% FBS. At 40-50% confluency, the cells were transfected with anti-EGFR antisense nanogenomedicine formulated with starburst polyamidoamine (PAMAM) dendrimers, Superfect®, as a non-viral gene delivery system. Cytotoxicity assessments and EGFR gene expression profiling were evaluated using MTT assay and semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) technique, respectively. The cytotoxicity assessments revealed insignificant ($p>0.05$) cellular toxicity by dendrimer alone and ODNs: superfect nanogenomedicnes. RT-PCR analysis resulted in 70% reduction in EGFR expression normalized upon expression of a house keeping gene (β-actin). The anit-EGFR ODN alone and scrambled antisense failed to inhibit EGFR expression indicating the effectiveness of the gene delivery system as well as EGFR antisence. Based on our findings, anti-EGFR antisense significantly inhibits EGFR transcriptome and may be considered as a promising adjuvant treatment strategy in breast cancer.

O-756
A CHOLINE OXIDASE BASED BIOSENSOR USING MULTIWALLED CARBON NANOTUBES MODIFIED GOLD ELECTRODE

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Carbon nanotubes have unique properties such as chemical stability, good mechanical properties and high surface area that make them ideal for the design of sensors. In this study, an amperometric choline biosensor based on the multi-walled carbon nanotubes modified gold electrode and choline oxidase (ChOx) was developed for the specific detection of choline. Amperometric detection of choline was carried out at 0.4 V (vs. Ag/AgCl) in 0.05 M phosphate buffer solution (pH 8) and room temperature. The performance of this biosensor showed a high sensitivity of about 74.6 micro-Amper/mM in determination of choline with a linear range from 2.5 X $(10)^{-7}$ to 7 X $(10)^{-7}$ M and a response time of 10 s. The detection limit of choline was determined to be about 3.2 X $(10)^{-8}$ M. These experimental results showed that carbon nanotubes are promising materials as electrochemical mediators and enzyme stabilizer for immobilization of enzyme in choline biosensor construction.

p-757
DETERMINATION OF KINETIC PARAMETERS OF CHOLINE OXIDASE ELECTRON TRANSFER BY PHYSICAL ABSORPTION ON MULTI WALLED CARBON NANOTUBES

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Choline oxidase (ChOx) was physically absorbed on the surface of the multi walled carbon nanotubes modified gold electrode. The immobilized ChOx indicated a bioelectrocatalytic activity for the oxidation of choline. The cyclic voltammetric results showed a well defined redox peak indicating the establishment of an effective and stable direct electron transfer reaction between the enzyme and electrode. The experiments were carried out in 0.05 M phosphate buffer solution pH 8.0. The results represented a formal potential, $E^{o\prime}$, of about 52 mV (vs. Ag/Ag Cl) which was almost independent on the scan rates. Based on the surface-controlled redox process equations, electron transfer coefficient and the electron transfer rate constant were estimated to be 0.36 and 1.6 s-1 respectively. These results indicated that carbon nanotuebs might be a suitable candidate among the various carbon materials for promoting the electron transfer reaction of choline oxidase.

p-758
INTERACTION OF TIO2 NANOPARTICLES WITH MICROTUBUAL PROTEIN

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High dynamic mitotic spindle microtubules are among the most successful targets for anticancer therapy. Exogenous ligands can disrupt the normal process by either increasing or decreasing microtubules stability and inhibiting their dynamic behavior. Nanoparticles are small enough to enter almost all areas of the body, including cells and organelles, potentially leading to a new approach to medicine (nanomedicine). Few studies have shown the application of titanium dioxide nanoparticles in the field of cancer treatment. Using UV spectrophotometer we have showed the effect of TiO2 nanoparticle on microtubule, in vitro. Our results revealed that TiO2 decreases microtubule polymerization. Intrinsic fluorescence spectroscopy results have showed significant changes in the structure of tubulin in presence of TiO2 naoparticles in a dose dependent manner. This study can be continued with the goal of developing new ways for cancer therapy with low cost drug.

p-759
ELECTRON TRANSFER KINETICS OF GLUCOSE OXIDASE USING CARBON NANOTUBES GROWN ON AN ALUMINA SUBSTRATE AS A BIOSENSOR

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Glucose oxidase (GOx) was immobilized on entangled and high surface area single wall carbon nanotubes (SWNTs) grown on an alumina substrate and direct electron transfer between GOx and electrode was studied. All cyclic voltammograms were recorded in a 0.05 M phosphate buffer solution at pH 7.0 and room temperature. The experimental results showed that GOx was adsorbed onto SWNTs/Alumina. While the immobilized enzyme was able to establish a direct electric communication with electrode, it showed a bio-catalytic activity towards glucose. The formal potential, E°, of GOx was measured to be 554 ± 2 mV (vs. Ag/AgCl). This value was independent of scan rates. The anodic to cathodic peak current ratios at different scan rates were close to one. This indicates that the electrochemical process of SWNTs modified electrode is quasi-reversible. Also the redox process was surface-controlled. Electron transfer coefficient and the electron transfer rate constant are estimated to be 0.35 and 0.34 s^{-1}, respectively.

p-760
NANO STRUCTURE MODELING AND COMPARATIVE GENOMICS OF L-ASPARAGINASE FROM DIFFERENT BACTERIA

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L-asparaginase is a therapeutic enzyme which is used as an anti leukemia enzyme in treatment of some cancers such as acute lymphoblastic Leukemia and non-hodgkin's Lymphoma. Although L-asparaginase from different sources has been studied and reported but only those from *Eshericshia coli* and *Erwinia chrysanthemi* are used as therapeutic anti cancers. In this study the nano structure of aparaginase (in 0.18 nanometer resolution) from five bacteria (*Escheichia coli, Erwinia crysanthemi, Pseudomonas 7A, Wolinella succinogenese* and *Acintobacter glutaminasificans*) studied and compared. There are four active sites in asparaginase tetramer, located in the interfaces between two monomers. Active sites are located close to the surface of the tetramer. Asparaginase is active as a homotetramer with molecular weight of ~140 kD. Each monomer consists of ~330 amino acid residues. No cooperativity or allosteric effects have been observed for this enzyme. Although the overall structure is similar but the nano structure anatomy of active site is different among them. For better understanding of these variations, the amino acid sequence of them were aligned and compared. Comparison of three dimension structure and protein sequence of the five mentioned asparaginases gave useful information about the mechanism of enzyme activity, substrate specificity and structural basis of its reported side effects. A motif of three amino acids important for substrate specificity and the side effects caused in patients treated with these enzymes was found.

p-761
CONSTRUCTION AND STABILIZATION OF SECRETORY CELL LINE EXPRESSING RECOMBINANT CAMEL SINGLE DOMAIN ANTIBODY LABELED WITH GFP USING SYNTHETIC LYSOZYME SIGNAL PEPTIDE

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There is an increasing interest in the application of nanobodies such as VHH in the field of therapy and imaging. In this study a stable genetically engineered cell line of Chinese hamster ovary (CHO) using an expression vector to permit extra cellular secretion of single domain antibody was prepared along with green fluorescent protein (GFP) as reporter gene. Fluorescent labeling of the proteins is a widely used method in biochemical, immunologic, and clinical studies. For a number of analytical applications fluorescent antibody fusion proteins offer advantages over the use of chemically labeled fluorescent antibody. In our experiments we have used a signal sequence containing a positively charged N-terminal region followed by a hydrophobic segment. These types of signal peptides were found to interact with membrane and facilitate the protein secretion. The quality of the construct was examined both by the restriction map as well as sequence analysis. The gene transfection and protein expression was

further examined by reverse transcriptase (RT-PCR). The transfected cells were grown in 200 µg/ml hygromycin B containing media and the stable cell line obtained showed fluorescent activity for more than a period of 180 days. The production of fusion protein was also detected by fluorescent microscopy, fluorescent spectroscopy as well as by enzyme-linked immunosorbent assay (ELISA) analysis during 6-months regularly. After this period, DNA was extracted from transfected cells and PCR results showed integration of fluobody gene. This strategy allows a rapid production of recombinant fluobodies involving VHH, which can be used in various experiments such as imaging and detection in which a primary labeled antibody is required.

p-762
PRODUCTION AND CHARACTERIZATION OF NOVEL CAMELID NANOBODIES AGAINST CD105

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Camelids produced functional antibodies that devoid of light chains and constant heavy chain domain (CH1). These unique antibody isotypes interact with the antigen by the virtue of only one single variable domain (VHH) and the crystal structure of an isolated VHH indicated that it is a particle of 2.5 nm in diameter and ~4 nm high, and has been referred to as a nanobody. These extremely stable, highly soluble nanobodies (VHH) which are expressed well in E. coli react specifically to antigen and have a close homology to human VH3 family. CD105, which is an accessory protein of the TGF-β receptor complex, is an attractive molecule for the targeting of the tumor vasculature. Up-regulation of CD105 on proliferating endothelial cells associated with tumor neo vascularisation. In this study, cloning the repertoire of the variable domains of IgG2 and IgG3 from immunized dromedary in pCom3X phagemid resulted in a large diverse library (10^7). The specific nanobodies were selected by five round panning using phage display technology. The single clone selections were carried out on output of forth and fifth round of panning and characterized by ELISA. The selected clones showed the good specificity and affinity to CD105. The antibodies obtained from these clones may be used for endothelial tumor cells targeting and angiogenesis inhibition.

O-763
PREPARATION OF POLY (VINYL ALCOHOL) NANOPARTICLES USING FREEZING-THAWING PROCESS

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Hydrogel nanoparticles are among the most promising nanoparticulate drug delivery systems. In this study poly (vinyl alcohol) (PVA) hydrogel nanoparticles have been prepared using water in oil emulsion technology plus cyclic freezing- thawing process. Effect of a series of methodological variables including polymer concentration, homogenization speed and time, types of different oils, hydrophilic/ lipophilic volume ratio, and particle separation method on the size and size distribution of resulting particles were evaluated. Finally, an optimized method has been established for preparation of nanoparticles with final mean particle size of 377.6 ± 1.15 nm. In this study, we have prepared hydrogel nanoparticles of PVA using a physical method based on freeze-thaw cycling. The preparation method has been optimized with respect to methodological variables in terms of their corresponding effects on particle size and particle yield. PVA solution was added to oil phase (silicon oil, caster oil or season oil) and the mixture was homogenized. The resulting nano-emulsion was then frozen for hours and allowed to thaw at room temperature. This cycle was repeated for three times. The nano-suspension prepared was extracted with chloroform and water. The aqueous phase was separated and used for particle size analysis. The results showed that PVA hydrogel nanoparticles possess smaller size and normal size distribution when season oil was used as organic phase. The PVA concentration of 10%, agitation rate of 14000 rpm in 5 min and volume ratio of aqueous phase to the organic phase 1:20 were found to be the optimum conditions.

p-764
BIOMIMETIC DESIGN OF PEROXIDASE AS AN ARTIFICIAL ENZYME

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The de novo design and redesign of artificial enzymes is an area of intense research focused on exploring the structure–function relationships of natural enzymes in pure studies or to develop industrial and medical applications as catalysts, sensors and molecular motors. Minimizing enzymes by simulating their active site is at the center of these studies, where the functional groups and the specific essential microenvironment should be assembled by no natural blocks. Peroxidase is nearly a simple enzyme having a heme group located in the hydrophobic pocket. Cytocrome C peroxidase (CCP) enjoys similar functional group so heme with its nearby amino acids can be obtained by proteolysis of CCP through pepsin digestion and manipulated to get the activity of the natural enzyme. Present study deals with the investigations on the kinetic parameters of microperoxidase in the presence of host nano-structured materials and optimization of conditions to achieve more efficient biocatalyst having effective synergy of the structure and function related to the evolutionary developed enzyme.

p-765
PREPARATION AND IN VITRO EVALUATION OF LINEAR AND STAR-BRANCHED PLGA NANOPARTICLES FOR INSULIN DELIVERY

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In the present article we have reported the synthesis and characterization of linear , poly (D, L- lactide-co-glycolide) (PLGA)- poly ethylene glycol(PEG-PLGA) and star-branched β- cyclodextrin- PLGA (β-CD-PLGA) and glucose- PLGA (Glu-PLGA) copolymers containing insulin as a model peptide drug. Linear and star-branched copolymers of PLGA were synthesized by bulk melt polymerization of the lactones (lactide and glycolide) in the presence of the PEG, glucose or β-CD using Sn-octoate as catalyst. Nanoparticles were prepared by a modified (w1/o/w2) double emulsion method. Bovine insulin was successfully encapsulated into the linear and star-branched PLGA nanoparticles with retention of insulin stability. The nanoparticles preparation process was optimized to reduce the burst effect and provide in-vitro sustained release. The average particle size of samples was 120- 355 nm. The cumulative amount of 65-84% insulin was released from the samples after 24 days. The yield of encapsulation of insulin was superior to 95 %. The in-vitro results suggest that the novel PLGA nanoparticles could be used as a carrier for prolonged delivery of protein-peptide drugs.

p-766
NANOTOPOLOGY OF THE HUMAN MYOCARDIUM MITOCHONDRIAL MEMBRANE: A PORPHYRIN TRAPPING SUBDOMAIN IN 17.6 KD α-NONBUNDLE PROTEIN

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Once the primary and 3D structures of the monomeric 17.6 kDa porphyrin IX (K)-signaling α-protein purified from the human heart muscle mitochondrial external membranes got revealed using a conventional X-ray crystallographic and SANS techniques, the nanotopology patterns for in situ porphyrin-protein affinity recognition comes to be next in line. Thus, a fluorescence resonance energy transfer (FRET) spectroscopy has been employed to resolve the Mag-Indo-1 and eosin induced fluorescence spectra in the both isolated whole mitochondria and pure receptor protein, either free or porphyrin-bound ones. As a result, a porphyrin trapping subdomain has been properly placed and elucidated of being the N-terminal hydrophobic α-helix related segment formed by H18, C24, H33 residues with the 4.6 nm Donor-Acceptor (H16-W15) energy transfer distance rang. The subdomain is found to be situated within a 5.2-5.8 nm membrane in-depth position neighboring to the membrane-crossing pore (channel). The nanotopology found looks clearly promising for further studies with respect to effects of porphyrins and their derivatives on the mitochondria function.

Protein Engineering

p-767
IDENTIFICATION, CLONING AND EXPRESSION OF PERSIAN MELON ALLERGEN

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Background: Allergy to melon (*Cucumis melo*) is one of the most frequent fruit allergies in Iran. Allergenic proteins (allergen) are responsible for sensitization of patients to allergenic materials. These allergens can also induce clinical symptoms in allergic patients. Identification and expression of these proteins could help in the diagnosis and therapeutic of allergic diseases. Objectives: The aims of this study were identification, cloning and expression of Persian melon allergens. Methods: Using sera from patients sensitive to Persian melon, western blotting was performed to identify, allergenic proteins. After extraction of mRNA from persian melon, and preparation of cDNA, using PCR, a fragment has been amplified. Complete gene has been sequenced after insertion into pET21b+ vector. The resulting vector was transformed into BL21 E. Coli. After expression and purification of the recombinant allergen, its homogeneity was confirmed by SDS-PAGE. Natural profilin was purified from Persian melon extract by immuno-affinity chromatography. Results: The identified, cloned and expressed allergen was profilin. The sequence of this protein had a length of 396 bp corresponding to 131 amino acid residues and a predicted isoelectric point of 4.46. The deduced amino acid sequence of the corresponding protein showed high homology with other plant profilins (71-84%) recently described as allergens. This protein has been expressed and purified by metal affinity chromatography. Recombinant profilin has been compared and showed similar properties to its natural form by skin prick test.

p-768
XPRESSION OF A GEMINIVIRUS SATELLITE DNA BETA ENCODED PROTEIN AND ITS TOMATO HOST PLANT INTERACTING PROTEIN IN THE BACTERIAL SYSTEMS

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Cotton leaf curl disease is caused by a combination of a satellite DNA, named DNA β, and one of a few species of Cotton leaf curl viruses (CLCuV). A complementary-sense ORF of CLCuV DNA β, βC1, is required for inducing disease symptoms and mediates the systemic spread of the virus. Using the yeast two-hybrid system, it has been shown that the βC1 protein interacts with a tomato ubiquitin conjugating enzyme, designated as SlUBC. To study the in vitro interaction of βC1 protein and tomato SlUBC, both proteins were separately expressed in two different bacterial systems.

Cloning of the CLCuV βC1 and ORF SlUBC ORF into the bacterial respective expression vectors pQE30 (QIAGEN) and pCAL-n FLAG (Stratagene), resulted in the production of His-tagged βC1 and CBP-tagged SlUBC fusion proteins, respectively. When compared to molecular weight markers, the βC1 and SlUBC fusion proteins migrated in SDS-containing gels to positions corresponding to a mass of approximately 14000 Da and 20000 Da, respectively. The specificity of the expressed His-βC1 and CBP-SlUBC fusion proteins was demonstrated by the binding of the respective proteins to the mouse anti-poly His and anti-FLAG monoclonal antibodies (Sigma) in immunoblot experiments, respectively. In vitro binding assays to confirm the interaction of CLCuV βC1 and SlUBC proteins are in progress.

p-769
INVESTIGATION OF A FLEXIBLE LOOP ROLE IN DETERMINATION OF LIGHT COLOR IN FIREFLY LUCIFERASE BY SITE DIRECTED MUTAGENESIS

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Firefly luciferase catalyzes the oxidation of substrate luciferin in the presence of ATP, Mg2+ and molecular oxygen. The product, oxyluciferin, is generated in an excited state, which then decays to the ground state with the emission of a photon. This enzyme efficiently converts chemical energy into light with a quantum yield of 0.88. Due to its high sensitivity to ATP, firefly luciferase is used extensively for measuring microbial contamination and for genetic reporter assay in molecular biology. Bioluminescence is characterized by a wide range of colors from green to red. A key question is the mechanism of light-producing color in luciferase reaction. Many investigations are performed about this subject by random and site directed mutagenesis. Many residues have been identified by these methods. In this research we studied the influence of insertion of additional residues in a loop (positions 250-259). Kinetic and structural effect of this insertion on luciferase properties will be reported.

p-770
DETERMINATION OF SENSITIVE BONDS TO PROTEASE DIGESTION IN FIREFLY LUCIFERASE USING DOCKING PROGRAM COMPUTATIONS AND BIOINFORMATICS TOOLS

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Firefly luciferase is a 62 KDa protein which catalyses the production of light in the presence of luciferin, Mg^{2+}-ATP and molecular oxygen. Firefly luciferase is widely used as a reporter in cell and molecular biology and has a broad range of applications in invivo imaging. Crystal structure of luciferase reveals that protein folded into two compact large N-terminal and C-terminal domains. The large N-terminal domain consists of three sub-domains A, B and C. One of its distinctive properties is it's pronounce susceptibility to proteolytic degradation which reduces its intracellular half-life. The purpose of this study was production of a resistant protein against proteolytic enzymes using site directed mutagenesis strategies. Limited proteolysis of photinus pyralis luciferase using trypsin showed that its cleavage at positions K206, R213, R218, K329, R330 and R337 which located in two accessible and flexible regions of sub-domains A and B. In attempt to elucidate the suitable cleavage site for digestion, bioinformatics studies were carried out. Consistent with bioinformatics studies, docking program computations showed that R337 may be critical residue in initial digestion.

p-771
MUTAGENESIS OF IRANIAN FIREFLY (*LAMPYRIS TURKESTANICUS*) FOR SCREENING OF NOVEL ENZYME BY SITE DIRECTED MUTAGENESIS

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Firefly luciferase catalyzes the oxidation of luciferin in the presence of ATP, Mg2+, and molecular oxygen. In nature, bioluminescence emission by beetle luciferases is observed in colors ranging from green (~530 nm) to red (~630 nm), yet all known luciferases use the same luciferin substrate. This enzyme efficiently converts chemical energy into light with a quantum yield of 0.88. Due to its high sensitivity and extreme specificity for ATP, luciferase has been used to determine the amount of ATP in various biological samples. Furthermore, luciferase is now being used in various applications, including food testing, BLI, BERT, bioluminescence immunoassay and for genetic reporter assays in molecular biology. However, there are several limiting factors for further application and development of this technology, including the low stability of the enzyme, a low turnover number, and a high Km for ATP substrate. A rational strategy for increasing the stability of a specific protein is by site-directed mutagenesis. In this study we made a set of red-shifted multicolored mutant by site directed rmutageneis in an Iranian luciferase (*Lampyris turkestanikus*). The mutated cDNAs were cloned in pET28a plasmid. After confirmation by sequencing, and protein expression we purified each mutant protein and calculated their kinetic properties. Bioluminescence emission spectra indicated that saturation at position 354 produce luciferases that emit light with various colors. The characterizations of mutants and the comparison of results with native luciferase showed that mutations of firefly luciferase resulted in the shift of the bioluminescence to the red region. The results of present investigation propose a speculative mechanism for color determination in firefly bioluminescence.

p-772
MOLECULAR CLONING AND EXPRESSION OF THE ARTHROBACTER VISCOSUS PENICILLIN G ACYLASE GENE IN PET21 VECTOR

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Introduction & objective: The excessive use of β-lactam antibiotics has led to the development of resistance in pathogens therefore newer semi-synthetic antibiotics are produced to overcome the resistance. Penicillin G and penicillin V are the natural penicillins produced in buik by fermentation. However, only a small amount of these penicillins is used directly as therapeutic agents while the majority is used for the production of 6-aminopenicillanic acid (6-APA). Penicillin G acylase (PGA; EC 3.5.1.11) is an important enzyme in pharmaceutical industry. This enzyme catalyzes the conversion of natural penicillins to yield phenylacetic acid and 6-aminopenicillanic acid. 6-APA is the β-lactam nucleus from which a wide range of semi-synthetic penicillins are made. This enzyme also converts cephalosporin to 7-aminodeacetoxycephalosporanic acid (7-ADCA). 7-ADCA is a precursor for all cephalosporins family. Penicillin acylase activity is present in a variety of microorganisms such as bacteria, filamentous fungi, and yeast. Gram negative bacteria such as *Escherichia coli*, *Alcaligenes feacalis* accumulate PGA in the periplasmic space and Gram positive bacteria such as *Arthrobacter viscosus* and B*acillus megaterium* produce extracellular PGA. Materials and Methods: At first genomic DNA from *Arthrobacter viscosus* (DSM NO. 20159) was extracted to prepare the template. Then proper and specific primers were designed for structural gene. The PCR product was cleaned up and ligated to the EcoRV-digested, and dephosphorylated vector pGEM-5zf (+/-). The construct transformed in to competent E.coli DH5α cells. For screening of recombinant clones the cells were grown on agar plate containing ampicillin, IPTG and X-gal. These clones harboring the gene for PGA were selected for plasmid extraction. Recombinant vector was doubly digested with NdeI and SalI. The insert was purified from agarose gel with high pure PCR product purification kit. Expression vector pET21 (a) was also doubly digested with NdeI and SalI and cleaned up. After ligation of extracted fragment and PET21 (a) we sent the recombinant vector for sequencing. This recombinant vector transformed to BL21 (DE3) E.coli, Pc-G acylase positive clones were isolated using the *Serratia marcescens* ATCC 27117 overlay technique because this strain is sensitive to 6-aminopenicillanic acid and resistant to Pc-G. The positive clones from overlay technique were grown in M9 minimal medium for expression of this protein. Result & conclusion: Electrophoresis of PCR product showed a single DNA fragment (2.4 kb) which corresponded to correct size of PGA structural gene of A.viscosus (2408 bp). Electerophoresis of reacombinant pGEM-5zf (3003bp) showed a size of 5.4 kb after PCR confirmation. Also electrophoresis of recombinant expression vector pET21a (5443 bp) showed a 7.8 kb fragment that the identity of PCR product was confirmed by digestion with NdeI/SalI. After sequencing we tried to express the recombinant protein. Plaques of *Serratia marcescens* ATCC 27117 confirmed the expression of this enzyme by transformed E.coli BL21 (DE3) and showed that PC-G is converted to 6-APA. Although a lot of the product was as an immature precursor, SDS-PAGE result confirmed induction of the expression vector.

p-773
COMPARATIVE ANALYSIS OF THE PROTEIN PROFILE OF WILD TYPE AND ACX4 MUTANT TYPE OF ASPERGILLUS

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Aspergillus fumigatus is an opportunistic human fungal pathogen, especially in immunocompromised individuals. The availability of the annotated A. fumigatus genome sequence will significantly accelerate our understanding of this organism. Recently three annexin genes have been described in this organism. In this study, one of these genes, ANXC4, was deleted using homologous gene disruption method. To better understanding of AXC4 function, we have analyzed the protein profile of both AXC4 deleted and wild type. Using 2 DE technique, preliminary data have shown obvious changes in mutant protein profile compared with wild type.

p-774
PHYSICOCHEMICAL, IMMUNOLOGICAL AND PHARMACOKINETIC IMPROVEMENT OF ANTI-LEUKEMIC ENZYME L-ASPARAGINASE BY CONJUGATION TO OXIDIZED INULIN POLYMER

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L-asparaginase (ASNase) is an important chemotherapeutic agent used for the treatment of variety of lymphoproliferative disorders, acute lymphoblastic leukemia in particular. Unfortunately, its use has been limited by short half-life and immune reaction. One approach to improve these characteristics is changing its physicochemical and biological properties by means of carbohydrate polymer conjugation. In this study we investigated the effect of conjugation with inulin on the physicochemical and immunological properties of L-ASNase. The methods used for the modification of enzyme involved periodate oxidation of inulin (MW~25000 D) and incubation with enzyme at 37 ºc for 5 days. The degree of ε-amino group modification was determined by TNBS method. The immunogenecity of modified enzyme was compared by means of SRID method between native and modified ASNase. Anti asparaginase sera were obtained from New Zeland rabbits after repeated injection of modified and native ASNase. A gentle periodate oxidation of inulin resulted in the highest residual enzyme activity (>80%). The Km (app) of glycoconjugate (1.6×10^{-3} M) was higher than the Km of native enzyme (0.74×10^{-3} M) which suggests that the affinity of enzyme to substrate L-asparagine was elevated. Modified ASNase was characterized by higher heat stability and more resistance to trypsin digestion, higher activity after exposing to sera than that of intact ASNase. Antibody titer was generally reduced in modified L-ASNase treated rabbit. Results suggest that inulin conjugation of ASNase may provide an alternative means to improve its effective use in therapeutics.

Signaling

p-775
THE INVESTIGATION OF TGF-β2 SIGNALING PATHWAY IN GM-CSF GENE UPREGULATION BY HUMAN BLADDER CARCINOMA CELL LINE 5637

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Transforming growth factor beta (TGF-β) family are multifunctional polypeptide factors, which have extensive inhibitory effects on cell growth and multiplication, and decrease transcription and mRNA stability for many kinds of growth factors like GM-CSF . Human bladder carcinoma cell line 5637 produces GM-CSF in an ectopic and paracrine status. To investigate the potential role of TGF- β_2 in GM-CSF production, 5637 cells were treated by 5 ng/ml of TGF- β_2. The results showed no anti proliferative activity of the factor on the recruited cells. However, ELISA, biological assay and Real-Time PCR along with Northern blot analysis demonstrated GM-CSF gene up regulation. To pursuit for the mechanism of TGF- β_2 signal transduction in GM-CSF gene modulation we evaluated the cells for PI3K isoforms expression. We sought for catalytic subunits P110α, β, δ (class I), $C_2\alpha$, C_2 β and $C_2\gamma$ (classII), C_3 (classIII), and regulatory subunits R_2 and R_3. Whereas the expression of P110α, β, δ, $C_2\alpha$, R_2 and R_3 isoforms was established, we could not show transcripts for C_2 β, $C_2\gamma$ and C_3 through 5637 cell line. Although most of the TGF- β signals are transferred by PI3K pathway, using wortmannin as a universal inhibitor for PI3K isoforms, our data showed PI3K was not the primary and principal pathway for the observed increase in GM-CSF gene expression. Unexpectedly relative quantitation of GM-CSF transcripts indicated that wortmannin behaved synergistically with TGF- β2 to upregulate GM-CSF gene expression.

p-776
THE EFFECT OF PI3K INHIBITION ON SECRETORY MMP'S GELATINOLYTIC ACTIVITY AND GENE EXPRESSION THROUGH GENITOURINARY PC3, DU145 AND 5637 CELL LINES

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MMP's (Matrix metalloproteinases) roles in invasion, metastasis and angiogenesis have been demonstrated. In particular, MMP-9 and MMP-2 play significant roles in cancer progression and metastasis. Since the over expression of molecules involved in tumor invasion and metastasis can be therapeutically targeted to disrupt the growth of malignant tumors, MMPs would be potential targets. It has been demonstrated that phosphatidyl inositol 3-kinase (PI3K) pathway is one of the major signaling routs in MMPs regulation. The abnormality of PI3K in many cancers including genitourinary ones has been evidenced. We examined human prostate cell lines PC-3 and DU-145, and 5637 human bladder carcinoma cell line for secretory MMP-9 and MMP-2 activities and their correlation with PI3K signaling pathway. Gelatinolytic zymography showed that specific blocking of PI3K pathway by wortmannin reduced MMP-9 activity in PC-3 cells media However, no alteration in MMP-9 was found in DU-145 cells, and wortmannin increased MMP-2 activity from 5637 bladder cells. Real-Time RT-PCR of MMP-9 and MMP-2 transcripts revealed that the decreased MMP-9 activity in PC-3 cells media is concomitant with decreased MMP-9 gene expression. However, PI3K inhibition did not contribute to gene expression for MMP-2 in 5637 cells. These observations suggest, while the PI3K activity contributes to tumor invasion, it's pharmacological or biochemical inhibition may equally culminate in the invasion of a subset of urogenital cancerous cells.

p-777
INVOLVEMENT OF PPAR-G AND P53 IN DHA-INDUCED APOPTOSIS IN REH CELLS

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Docosahexaenoeic acid (DHA, 22:6 n-3) is an omega-3 polyunsaturated fatty acid that is found in fish oil and exerts cytotoxic effect on a variety of cell lines. The molecular target responsible for mediating the effect of DHA remains unknown. The objective of the present study was to present experimental evidence for the role of PPAR-gamma in conveying the cytotoxic effect of DHA. DHA induced apoptosis in Reh and Ramos cells and apoptotic effect of DHA was inhibited by the PPAR-gamma antagonist GW9662, indicating that PPAR-gamma functions might mediate the apoptotic effect of DHA. Furthermore, the study showed that DHA induced the PPAR-gamma protein levels in both Reh and Ramos cells. Moreover, DHA induced the expression of p53 protein in Reh cells in a PPAR-gamma-dependent manner. The up-regulation of p53 protein by DHA kinetically correlated with the activation of caspase 9, caspase 3 and induction of apoptosis, suggesting a role for p53 in DHA-mediated apoptosis in Reh cells. Taken together, these findings suggest a new signaling pathway, DHA-PPAR-gamma-p53, in mediating the apoptotic effect of DHA in Reh cells.

p-778
DOCOSAHEXAENOIC ACID SENSITIZES RAMOS CELLS TO RADIATION INDUCED APOPTOSIS THROUGH PPAR-GAMMA AND NF-KAPPAB INHIBITION

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Gamma irradiation (gamma-IR) resistance is a character of many malignant cells that decreases the efficacy of radiotherapy. A well recognized cellular response to low dose of radiation, in p53 -mutant cell lines, is the activation of the nuclear factor kappa B (NF-kappaB), which can lead to the inhibition of radiation-induced apoptosis. Thus, the inhibition of NF-kappaB activation is an important strategy to abolish radio-resistance. It has been shown that p53-mutant Ramos cells are highly resistant to radiation induced apoptosis. Moreover, many reports described that the activation of PPAR-gamma as a ligand-dependent nuclear receptor, can lead to inhibition of NF-kappaB. The objective of the present

study was to examine wether DHA increases radiosensitivity through the activation of PPAR-gamma and the inhibition of NF-kappaB. Ramos cells, which express wt PPAR-gamma, were co-treated by DHA and subjected to gamma-IR in absence and presence of PPAR-gamma antagonist (GW9662). DHA significantly increased radio-sensitivity in Ramos cells. Western blot analysis revealed that radio-sensitizing effect of DHA correlated with NF-kappaB inhibition, and pretreatment of Ramos cells with GW9662 prevented this process. The findings suggest that DHA may modulate NF-kappaB activity, and sensitize Ramos cell to gamma-IR through the activation of PPAR-gamma. They also show that DHA disposes Ramos cells to radiation- induced apoptosis via PPAR-gamma and inhibition of NF-kappaB signaling. These results suggest that combination therapy PPAR-gamma agonists and gamma-IR may increase therapeutic efficacy of radiation in tumors with non-functional p53.

p-779
CELL MIGRATION IS MODULATED BY INTEGRIN-FILAMIN LINKAGE

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The process of cell migration is crucial for many normal and pathological processes including embryo formation, wound healing, tumor metastasis. Migration can be divided into several consecutive steps: cell polarization and formation of protrusions, focal adhesions and cell retraction. The precise mechanisms by which focal adhesions are formed in migrating cells are a major challenge for modern molecular biology. This process largely depends upon the contact of extracellular matrix and the cytoskeleton through the integrins, the major family of migration-promoting receptors. The objective of the study was to investigate how actin-binding protein filamin A contribute to integrin signal transduction. Filamins contain 24 immunoglobulin-like domains of which 19-21 domain interacts with the cytoplasmic tails of integrin beta subunits. Recently, a splice variant of filamin A domain 19-21 variant 1 was found which enhances the binding ability of filamin to integrins. We are investigating whether the overexpression of filamin domains 19-21 affect cell morphology and integrin-mediated migration. Investigations have shown that pEGFP-FLN19-21 var1 affects the cell polarity, thus, causing the appearance of multiple protrusions. This prevents the efficient cell spreading. The preliminary suggestion is that enhanced filamin-integrin linkage controls the cell migration. Above research helps to establish the role of filamin variants in the properties of migrating cells.

O-780
INVOLVEMENT OF ERK/MAPK PATHWAY IN MEGAKARYOCYTIC DIFFERENTIATION OF K562 CELLS INDUCED BY 3- HYDROGENKWADAPHNIN

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Since differentiation induction therapy represents an attractive strategy for the treatment of a wide range of malignancies, universal efforts have been devoted to find new and potent differentiation inducers devoid of general toxicities. The aim of the study was to show that 3-Hydrogenkwadaphnin (3-HK), a novel daphnane-type diterpene ester isolated from Dendrostellera lessertii (Thymelaeaceae), was an effective inducer of megakaryocytic differentiation in chronic myelogenous leukemia (CML) K562 cell line based on the morphological features, expression of cell surface marker glycoprotein IIb (GPIIb), nuclear polyploidy and cell-substrate adhesion. The inhibition of cellular replication and the induced maturation produced by drug appears to be a consequence of activation of ERK/MAPK signaling. Inhibition of MEK activation by PD98059 reverses the growth arrest, decrease adhesive properties and block the expression of GPIIb integrin, induced by 3-HK. Immunoblot analyses showed that 3-HK induced sustained activation of ERK1/2 at early exposure times before the onset of differentiation and followed for 24-72 h. These results support that the ERK/MAPK signaling complex is regulating the action of 3-HK on cell cycle arrest and the program(s) that leads to differentiation toward megakaryocytic lineage.

p-781
STUDY ON THE MECHANISM OF CELL DEATH INDUCED BY RECOMBINANT SHIGA TOXIN (RSTX)

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Shiga toxins (Stxs) are a family of bacterial protein toxins produced by Shigella dysenteriae type 1 and Entero-hemorrhagic Escherichia coli (Stx-producing E. coli). These toxins are composed of one enzymatically active A subunit and five B subunits which carry the binding property of holotoxin to receptor molecules. Stx has been thought to induce cell death by inhibition of protein synthesis. This study investigated the cytotoxic effect of recombinant Stx on HeLa cells. The Stx-induced cell death was assessed in the presence of some inhibitors, including Ca^{2+}, protein G, and p38MAP kinase inhibitor. The preliminary results indicated that inhibition of intracellular Ca^{2+} has an effect on the cytotoxicity induced by recombinant Stx. Therefore, Ca^{2+} signaling is one of the important factors in recombinant Stx-mediated cell death.

p-782
EXPRESSION PATTERN OF FOCAL ADHESION KINASE (FAK) IN HUMAN ENDOMETRIUM DURING THE MENSTRUAL CYCLE

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Endometrial remodeling occurs during each menstrual cycle in women. It has been shown that, in a variety of cell types, the

processes of proliferation, signaling complex formation, and extra cellular matrix (ECM) remodeling require a cytoplasmic tyrosine kinase, focal adhesion kinase (FAK). The objective of the study was to examine the expression pattern of FAK in human endometrium during the menstrual cycle. Formalin-fixed paraffin-embedded endometrial samples from 54 pre-menopausal non-pregnant women undergoing hysterectomy and biopsy for benign diseases were subjected to antigen retrieval, immuno-stain with monoclonal antibody against FAK and ABC staining. Primary antibody was omitted as negative control and positive immunoreactivity in different sections was scaled. Immunoreactivity was assessed in luminal and glandular epithelium, endothelial and stromal cells. Early and late proliferative phases showed similar moderate staining in all cell types. Mid proliferative phase showed strongest expression of FAK, specifically in luminal and glandular epithelium and endothelial cells. Early and mid-secretory phases showed moderate expressions of FAK in all cell types. Late secretory and menstruation phases showed weak expressions of FAK in all cell types. This study has shown that endometrial FAK expression is a phase-dependent manner during the menstrual cycle. It appears that FAK plays a critical role in endometrial remodeling, and its activity may be regulated by steroid hormones.

p-783
STRATEGIES FOR CHOOSING INHIBITORS OF RAS PATHWAY

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In cancer cells dys-regulated cell signaling may occur through over-expression or mutations of proto-oncogenes. One of such proto-oncogenes is Ras group, which is the most frequently detected oncogene in human malignancies. RAS activation leads to signaling through a lot of pathways. Because of high percentage of tumors harboring oncogenic Ras, interrupting the Ras-signaling pathway has been a major focus of new-drug-development efforts. Controlling upstream of Ras-signaling pathway is ineffective since positive and negative regulators can affect the strength and duration of Ras-signaling. In addition, Ras proteins play central roles in controlling signal pathways in normal cells. Thus, Ras inhibition is detrimental to normal cells. Therefore the best way for controlling cells with mutant k-Ras is the inhibition of its far effective downstream targets. Mutant Ras affects a complex set of transcriptional targets. These targets can be identified by subtractive suppression hybridization (SSH), northern blot and microarray. We studied up-regulated genes (at least 50 fold) downstream of mutant k-Ras like anexin IV, mammalian protein, exportin, MMP collagenases (MMPs), VEGF, tyrosine phosphatase-like protein. The study identified two candidates for the inhibition as they had a pivotal role for cancer metastasis, and were more downstream than others. The first one was VEGF, which was upregulated in many cancers with mutant k-Ras. Inhibitors of MAPK, PI3K -AKT and c-myc can regulate its expression. MMPs (MMP2, 9), which increased tumor cell motility and metastasis was the other target. Inhibitors of Ras-ERK and PI3K-AKT pathways can block this effector of Ras. The above mentioned approaches are preferred as they are less toxic for normal cells have priorities.

p-784
THE ROLE OF GαQ ON β-CATENIN LOCALIZATION AND FUNCTION

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b-Catenin, a component of Wnt signal transduction, plays a key role in various cellular events such as differentiation, migration, and proliferation. In the nucleus, the interaction of b-Catenin with transcription factors like members of TCF and LEF family leads to the expression of several genes which encode very important proteins involved in the regulation of cell growth and proliferation. Deregulation of b-Catenin function occurs during formation of many human cancers; therefore, targeting b-Catenin could prevent and/or treat many human cancers. The objective of the study was to study the role of Gaq on b-Catenin function using mini-gene, which encodes a short peptide that inhibits Gaq specifically. A mammalian expression vector carrying the mini-gene was transfected into Human embryonic 293T cells by calcium phosphate. The efficiency of transfection was measured by flowcytometry via an expression vector encoding Green Fluorescence Protein, GFP. We have used immunofluorescence microscopy and Luciferase gene reporter assay to examine b-Catenin cellular localization and transcriptional activity. Our preliminary results show that the inhibition of Gaq has no effect on endogenous b-Catenin membrane localization. However, the expression of the Gaq inhibitory peptide significantly reduced cellular accumulation of exogenous b-Catenin. The inhibition of Gaq also decreased transcriptional activity of b-Catenin by two folds. These results suggest that Wnt signal transduction might function through Gaq class of G proteins to regulate b-Catenin cellular localization and function.

p-785
PIVOTAL ROLE OF CASPASES IN P21 CLEAVAGE AND STRESS MAPKS ACTIVATION DURING 3-HK-INDUCED APOPTOSIS OF U937 CELLS

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Despite the depth of knowledge concerning the pathogenesis of acute myeloblastic leukemia (AML), long-term survival remains unresolved. Therefore, new agents that act more selectively and more potently are required. A novel diterpene ester, 3-hydrogenkwadaphnin (3-HK), is able to induce both differentiation and apoptosis in various leukemia cell lines. After 3-HK (15 nM) treatment, a portion of U937 cells remained in suspension and underwent apoptosis, as confirmed using sub-G1 peak and Annexin-V/PI double staining. The kinetics of caspases activation in the drug-treated U937 cells showed that an increase in activities of caspase-8

and -3 at 12 h were followed by an increase in the activity of caspase-9 at 36 h. According to colorimetric assays, when U937 cells were co-treated with caspase-9 inhibitor and 3-HK, the increase in caspase-8 and -3 activities also began at 12 h, suggesting that the apoptotic mechanism was amplified via the involvement of the mitochondria. More detailed investigations revealed that both p21 cleavage and activation of stress MAPK pathways (JNK1/2 and p38) were also occurred among apoptotic cells. Moreover, caspase inhibitors impeded p21 cleavage and JNK1/2 and p38 activation. Therefore, caspases activities are required for 3-HK-mediated apoptosis, p21 cleavage and JNK1/2 and p38 activation. This novel signaling pathway and the efficiency of 3-HK may be useful to improve therapeutic options in AML.

p-786
MOLECULAR CLONING AND EXPRESSION OF RAINBOW TROUT (ONCORHYNCHUS MYKISS) INTERFERON REGULATORY FACTOR 7

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Rainbow trout interferon regulatory factor 7 (IRF7) gene was cloned from a subtractive cDNA library constructed with mRNAs which contains an ORF of 1249 nucleotides that translates into a 416 residues putative peptide. Three groups (n=5 each) of rainbow trout weighing (122 ± 5.5 g) were injected intramuscularly with a DNA vaccine containing the full coding sequence of the viral haemorrhagic septicemia virus surface glycoprotein G inserted into pCI neo-mammalian expression vector, poly I/C or phosphate buffered saline. Stimulation of embryonic rainbow trout cell line (RTS11) by poly I/C was also undertaken in vitro. Results showed that interferon regulatory factor 7 gene was expressed in kidney head, spleen and gill of fish 10 days after vaccination. There was also a significant increase in IRF7 expression level in kidney head of fish injected by poly I/C when compared with phosphate buffered saline receiving group. IRF7 transcript was up-regulated by stimulation of RTS11 by poly I/C in vitro.

p-787
GH3/B6 CELLS EXPRESS DECTIN-1 IN RESPONSE TO β-GLUCAN

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Glucans are the structural cell wall polymers of many fungi, plants and certain bacteria, which possess immunomodulatory activities. Previous studies indicated that such polymers were the active parts of some lactogenic plants. The objective of the present study was to examine whether GH3/B6 cells, rat pituitary cell lines that secrete prolactin and Growth hormones, express dectin-1 in the presence of β- glucan, which is a mammalian cell surface receptor which binds to (1→3)-β-D-glucan. Total RNA was extracted from spleen and GH3/B6 cells in the presence of β-glucan (250 μg/ml). Afterwards RT-PCR and PCR methods were applied to verify the expression of Dectin-1. Dectin-1 was expressed in GH3/B6 cells at a very low level, and the expression was intensified by β-glucan. Therefore, β-glucan may induce GH3/B6 cells throughout Dectin-1, which lead increasing prolactin secretion from these cells.

p-788
CAN PPAR GENE EXPRESSION BE INDUCED BY ACTIVATION OF PI3KINASE IN LIVER CELLS?

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It has been reported that 4HL can activate PI3K and increase insulin secretion. It has also been reported that PI3K activation can increase PPARγ gene expression. PPARγ is one of the most important ligand dependent transcription factors. It increases insulin secretion probably via an increase in the amount of insulin mRNA. PPARγ in normal hepatic cells regulates genes responsible to glucose homeostasis such as GLUTs and hexokinase. Such a pathway in hepatic cells helps a new anti-diabetic agent decrease liver cell resistance to insulin beside its effect in insulin secretion in β-cells. It was the aim of the present study to examine the effect of 4-HL on PPARγ gene expression in a hepatoma cell line HepG2 by western blot technique. In brief HepG2 cells were grown in 50 mm plates and incubated with different concentration of 4HL for 48 hrs. After cell lysis and protein determination (by multiwell Bradford protein assay) western blot was performed by a set of specific antibody against PPARγ. The findings show that 4HL could not change PPARγ gene expression. This shows that the cooperation between PI3K and PPARγ gene expression in liver cells is different from other cells. Our data is consistent with an in vivo study showing 4HL could not activate PI3K in rat liver while could increase insulin secretion.

4
Other Subjects

p-789

A STUDY ON ANTITETANUS TOXOID ANTIBODY TITER IN CHRONIC HEMODIALYSIS PATIENTS-SHIRAZ-1385

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Patients with ESRDx have higher incidence of infectious Dx which is thought to be related to impaired immune response, so immune response of H.D patients to vaccination is also impaired. Very few studies have focused on tetanus toxoid immune state in HD patients . Therefore a cross-sectional study was conducted on a total of 57 patients (54.4% males, 45.6% females) who were on H.D due to ESRD in Sahraee center of Shiraz in 1385, and 36 subjects as control group (44.4% males,55.6% females). The patients were under H.D due to CRF at least for 3 months and did not receive any antiT.T vaccine or I.G in the previous year. The control group had neither renal dx nor a history of antiT.T. vaccination or I.G. in the last year. Although their past history of vaccination was not reliable so the control group was chosen among patients' relatives. Groups were matched based on age (control mean age,52.8; & patients' mean age, 56.1 years) and sex as much as possible. The serum antiT.T IgG was measured by ELISA method. Some contributing factors such as sex, age, Hb and albumin concentrations ,duration of dialysis , number of H.D. per week, smoking, erythropoeitin or Venofer injection , socioeconomic status, height, weight , P.M.Hx , underlying renal dx , and major dx. were taken into account. The results were as follows: the mean serum IgG level of patients was 0.164 (+/- 0.322) & that of controls was 0.573 (+/- 1.13). Therefore 78.9% of the patients needed basic immunization, while 15.8% will be in the control group after 1-2 year & 5.3% after 2-4 years. On the other hand 52.8% of the control group needed basic immunization, 30.6% after 1-2 years, and 16.7% after 2-4 years. Meanwhile none of the contributing factors mentioned above seemed to be a reliable factor correlating reversibly with IgG level. Although reverse correlations of age (p. 0.091), H.D duration (p. 0.289), Height (p. 0.848) and venofer injection (p. 0.404) with IgG level, were detected, but they were not meaningful statistically. Moreover albumin (p.0.997), Hb (p. 0.856), weight (p.0.533), number of H.D per week (p.0.391) and erythropoietin (p.0.852) injection did not show any relationship with IgG. It is noticeable that of 6 patients with DM, as the underlying dx, 5(83.3%) needed basic immunization. This rate is 92.9% in Htn patients (13 out of 14) & 66.7% (8 out of 12) in patients with both diseases. 78.9% of the patients and 52.8 % of the control group were recommended for basic immunization.

p-790
EVALUATION OF THE EFFECT OF QUERCETIN ON PLASMA LIPIDS AND LIPOPROTEINS IN CHOLESTEROL FED-MAIL RABBITS

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Introduction: A most common cause of mortality in the West and other industrial communities is vascular disease induced by atherosclerosis. Different studies show that atherosclerosis is a senile illness, but under special clinical circumstances such as high plasma cholesterol, high blood pressure, smoking, diabetes mellitus and family history early atherosclerosis takes place. On the other hand, it has been shown that quercetin causes improvement of blood flow and decline of vascular atherosclerotic symptoms. In this research we studied the effects of quercetin on plasma lipids and lipoproteins in aorta of cholesterol fed-male rabbits. Methods: 32 male rabbits were used and divided into 2 groups as follows: group 1, cholesterol rich diet (CRD) + quercetin and group 2, CRD (control group). The animals received the diet and/or quercetin for 35 days. Blood samples were obtained before and after the test. Plasma cholesterol, triglycerides, LDL and HDL were then measured. Results: The experiments showed e significant decreases in cholesterol and triglycerides in the group receiving quercetin compared to the control group. Also the LDL and LDL/HDL ratio decreased in quercetin fed group compared to the control. In addition, there was a significant increase in HDL level of the quercetin fed groups. Conclusion: It is demonstrated that quercetin feeding has a reductive effect on plasma cholesterol, triglycerides and LDL concentrations and an enhancing effect on plasma HDL concentration.

p-791
CYSTEAMINE AS AN ANTIOXIDANT INFLUENCES IVM RATE, GSH LEVEL AND SPINDLE AREA IN MOUSE OOCYTE

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The present study was carried out to investigate the effects of different doses of cysteamine on the rate of IVM and to observe its effect on glutathione (GSH) synthesis without cumulus cells. For quantification of shape and size of microtubules, MII spindle area was analyzed. Female mice were primed with 5 IU of PMSG and GV oocytes were retrieved from the ovary 48 hr later for IVM. The IVM medium was supplemented with 0, 50, 100, 200 and 500 mM cysteamine. Experiments also included a group of ovulated oocytes (in vivo matured) after priming with PMSG and HCG. Cytoplasmic GSH level was measured by DTNB-GR recycling protocol. MII oocytes were fixed and immuno-stained for microtubules, and chromosomes, and the spindle area was analysed. After IVM of mice oocytes, an improvement was observed on MII development in 100 mM cysteamine. Intracytoplasmic GSH level increased in the presence of 100mM cysteamine. Highest level of GSH was produced in the in vivo group. Spindle area in all in vitro groups except the 500 mM cysteamine group increased compared to the in vivo group. Spindle area in 100 mM cysteamine group was close to that of the in vivo ones ($P>0.05$). Our results showed that cysteamine improved IVM rate in a dose dependant manner. Also cysteamine induced glutathione synthesis in MII oocytes and improved microtubule organization in the 100mM cysteamine group.

p-792
EVALUATION OF THE ROLE OF PLASMINOGEN/PLASMIN SYSTEM IN ANGIOGENESIS BY ANTIPLASMINOGEN MONOCLONAL ANTIBODIES

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Angiogenesis, the formation of new blood vessels from existing vasculature, is involved in normal development but also in a variety of pathologic conditions such as cancer, atherosclerosis and ocular diseases. Activation of fibrinolytic system is essential in angiogenesis process. Glu-plasminogen, the inactive precursor of plasmin, is the most important component of fibrinolytic system. New information about the steps that participate along the angiogenesis pathway, and the molecules responsible for these events, has led to a variety of novel, and increasingly mechanism-based, approaches for the development of angiogenesis inhibitors. Among these approaches, monoclonal antibodies, as effective biological molecules, are very important. In this study we evaluated the role of plasminogen/plasmin system in angiogenesis by two antiplasminogen monoclonal antibodies namely A1D12 (against the N-terminal domain of Glu-plasminogen) and MC2B8 (against the C-terminal domain of Glu-plasminogen). For this purpose, we set an in vitro angiogenesis model with cytodex-3-microcarrier attached endothelial cells and studied the effect of antibodies on angiogenesis process in the presence of controls. Results showed that MC2B8 inhibits angiogenesis. In contrast, A1D12 antibody activates in vitro angiogenesis.

p-793
EFFECT OF FOLLICLE STIMULATING HORMONE AND OTHER PARAMETERS ON IN VITRO GROWTH AND MATURATION OF MOUSE OOCYTES

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Progress in the treatment of human and animal infertility is directed towards the development of effective culture conditions for the in vitro maturation of oocytes. Mouse is the only species in which whole process of oocyte maturation has been successfully performed. The major endocrine factors affecting the ovarian oocyte growth are gonadotrophins, namely follicle stimulating hormone (FSH) and luteinizing hormone (LH). Ascorbic acid, serum and the amount of oxygen (supplied during in vitro maturation) also induce in vitro maturation of mouse oocytes. For the present study, intact preantral follicles were isolated from the ovaries of 6 week-old female Syrian mice using 25-gauge needles and cultured in TCM-199 medium supplemented with sodium pyruvate (2mM), glutamine (2mM), penicillin G (75µg/ml) and streptomycin (50µg/ml). Following analyses were performed: I) 10, 25, 50 and 100 IU/L of FSH were added to experimental plates containing 25-30 oocytes and 100 IU/L showed maximum effect on the growth of oocytes ($P < 0.05$). II) 5, 10, 20, 30, 40 and 50 IU/L of LH were used as above and 10 IU/L showed maximum growth effect ($P < 0.05$), III) fetal calf serum (1, 2, 3, 4, 5 and 10%) was added to separate plates containing the oocytes and 5% FCS was found to be an appropriate concentration ($P < 0.01$) and IV) 10, 20, 30, 50, 100, 200 and 300 µmol/L of ascorbic acid were analyzed in the presence and absence of fetal calf serum and 30 µmol/L of ascorbic acid was effective in the presence of FCS as compared to 50 µmol/L in the absence of 5% FCS ($P<0.05$).

p-794
EFFECT OF LIF ON LOW MOTILITY SPERMS IN MEN WITH ASTHENOSPERMI

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Introduction: From the past until now extensive study has been undertaken to deal with the infertility problems in men. Some of the causes related to mail infertility can be defined as reduced number of sperms, abnormal morphology, and weakness in the motility. Improvement in the quality of sperm such as number and morphology has not been accomplished by in vitro experiments at the present time. Improvement in quality of sperm motility by means of drugs is possible. Investigations have shown that a close relation exists between sperm motility and fertility rate upon in vitro addition of progesterone, platelet activating factor, follicular fluid, cytokines and pentoxyphilline resulting in the increased sperm motility and survival time. On the other hand, some studies have been done on the effect of LIF on normal sperm motility and have demonstrated that this factor could improve normal sperm motility. Since there is no report on the effect of LIF on low motility sperms in men with asthenospermi, we decided to determine the effect of this factor on low motility sperms. Materials and Methods: In this investigation the semen samples of 15 infertile men referred to IVF department of Imam Khomeini Hospital were collected and prepared for successive stages of culturing in Ham's F-10 medium with different concentrations of 3, 5, 10, and 50ng/ml LIF in an incubator at 37° C with 5 % CO_2 for periods of 6, 24 and 48 hr to evaluate motility and survival time. The data were statistically using ANOVA & LSD statistics by means of SPSS software. Results: The results showed that this factor does not affect the motility and survival time of the sperm after 6 hr, but after culturing for 24 hours in 10ng/ml and 48 hours 50ng/ml LIF, the results show improvement in both motility and vitality of sperms. Conclusion: our data showed that LIF has positive effects on motility and vitality of human sperm in a dose dependant manner.

p-795
EVALUATION OF COAGULATION AND FIBRINOLYTIC DEFECTS IN DIFFERENT DISEASES IN DOG AS AN ANIMAL MODEL

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Coagulation disorders reported in different diseases are caused by increased fibrinolysis and coagulation factor deficiency. During an investigation, citrated blood samples were collected from 215 dogs, as an animal model, with different diseases including bacterial and viral infections, hepatic failure, renal failure, cardiovascular diseases, gastrointestinal disorders, diabetes, neoplastic defects, anaphylactic and septic shock and trauma during and after surgery, DIC, and poisoning with anticoagulant rodenticides. All diseases were primarily confirmed with clinical observations and at least two paraclinical tests. Blood plasma were analyzed for activated partial thromboplastin time (APTT), prothrombin time (PT), fibrinogen (Fib) and total protein (TP). Dogs with infectious diseases had significantly higher APTT ($p < 0.047$) and Fib ($p < 0.001$). After surgery, significant prolonged APTT appeared ($p < 0.001$). Dogs with urinary disorders had significantly higher PT ($p < 0.03$). The most significant changes were the increased concentration of fibrinogen in gastrointestinal disorders with diarrhea and infectious diseases ($p < 0.000$). This study revealed that different disorders result in some changes in haemostatic condition of blood. Although they are not very serious, but they increase pathogenicity. So it is important to apply necessary treatments on time to prevent long lasting diseases and their sequelae including coagulopathy, fibrinolysis and DIC.

p-796
FREQUENCY OF COMMON ALPHA-1-ANTITRYPSIN VARIANTS FREQUENCY IN IRAN

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Alpha-1-antitrypsin (AAT) is the major inhibitor of neutrophil elastase. AAT deficiency (AATD) is accompanied by lung disease, some hepatic disorders, rheumatoid arthritis, glomerulonephritis and other disorders. AAT has a wide protein variant spectrum. M1, M2 and M3 are the most common normal alleles but the most prevalent alleles that cause AAT deficiency are S and Z variants. The allele frequencies of AAT variants have been identified in many countries but there was no statistical report in Iranian population. The aim of this study was to investigate the frequency of M1, M2, M3, S and Z variants in Iran. In this study 318 sera were obtained from healthy volunteer dormitory students of Tehran universities using ethnic stratified sampling. Then phenotyping was carried out by isoelectric focusing (IEF) with pharmalite pH= 4.2-4.9 in comparison with standard phenotypes. From 318 normal sera, 201, 55, 41, 8, 6 and 7 had M1, M2, M3, MS, MZ and other phenotypes, respectively. Allele frequency of M1, M2, M3, S, Z and other variants of AAT in the population of Iran were 0.6477, 0.1776, 0.1305, 0.0126, 0.0094 and 0.0220, respectively.

O-797
SERUM ALPHA-1-ANTITRYPSIN EVALUATION: A COMPARATIVE STUDY

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Alpha-1-antitrypsin (AAT) is the major component of human plasma alpha-1 electrophoretic band proteins and acts as inhibitor of proteolytic enzymes. AAT deficiency is accompanied by lung and liver disorders so using a precise and reliable method for its evaluation is important diagnostically. In present study 318 normal sera were obtained from volunteer students of Tehran Universities and serum AAT was evaluated by Cellulose Acetate Electrophoresis (CAE), Trypsin Inhibitory Capacity (TIC) and Single Radial Immunodiffusion (SRID) methods. 34, 84 and 112 sera were abnormal by TIC, SRID and CAE methods, respectively. 201 sera were normal as compared with reference range of CAE and TIC methods, 29 sera were abnormal by both methods, 83 sera were normal by TIC and abnormal by CAE and 5 sera were abnormal by TIC and normal by CAE. Also 227 sera were normal by TIC and SRID methods, 27 sera were abnormal by both methods, 57 sera were normal by TIC and abnormal by SRID and 7 sera were abnormal by TIC and normal by SRID. CAE and SRID methods as compared with TIC showed sensitivities of 70%, 83% and specificities of 85%, and 90%, respectively. Cellulose Acetate Electrophoresis and alpha-1 globulin band determination is routine in clinical laboratories, however it is not reliable. Although the SRID method is more sensitive and specific than CAE method, its sensitivity and specificity is lower than TIC method. Therefore TIC method is recommended as a precise and reliable method for serum AAT evaluation.

p-798
TELOMERASE ACTIVITY IN CEF CELL CULTURE INFECTED WITH DIFFERENT PASSAGES OF VERY VIRULENT MAREK'S DISEASE VIRUSES

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Telomerase is a ribonucleoprotein, which adds telomeric repeats onto the 3` end of existing telomeres at the end of the chromosomes in eukaryotes. There is a hypothesis that telomere length may function as a mitotic clock, therefore expression of telomerase activity in cancer cells may be a necessary and an essential step for tumor development and progression. In this study telomerase activity in chicken embryo fibroblast (CEF) cells infected with different passages of Marek's disease virus (an avian tumor virus) including woodlands P14, P80/1, P120, MPF57.P9 was detected using TRAPEZE® telomerase detection kit. Results showed that telomerase activity was present 5 days post-infection with the Woodlands strain at passage 14 and with the MPF57 strain at passage 9, and produced typical signs of Marek's disease in susceptible chicken. However, no telomerase activity was detected with higher passages of the Woodlands virus.

p-799
ENHANCED LIPID PEROXIDATION AND IMPAIRED ANTIOXIDANT BALANCE IN PATIENTS WITH CORONARY ARTERY DISEASE PROVEN ANGIOGRAPHICALLY

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The excess risk for cardiovascular disease is not explained by traditional risk factors and therefore oxidative stress has been proposed as a possible contributor for the increased risk of morbidity and mortality. The aim of this investigation was to evaluate the plasma indices of oxidative stress in patients with coronary artery disease (CAD) presented with myocardial infarction (MI) or without MI. As a comparison a group of healthy subjects was employed as control. This study was comprised of three groups including thirty eight CAD patients without MI, twenty CAD patients with MI and fifty five healthy subjects as control. Age ranges were 41-70, 41-68, and 39-69 years, respectively. Vitamin E concentration was determined by HPLC. Glutathione and malondialdehyde concentrations were estimated spectrophotometrically. Malondialdehyde levels were markedly higher in the patients

than in the controls (P<0.05). Lower glutathione levels were seen in patients compared with controls, while reduced vitamin E was only noted in CAD patients without MI (P<0.05). No differences were seen between measures of oxidative stress in patient groups. We have shown increased oxidative stress in CAD patients as a whole compared with controls, but no differences were seen when the patient group was divided according to the presence or absence of MI. This finding reveals that increased oxidative stress may precede the development of the clinical manifestations of CAD. Attenuation of glutathione and enhancement of lipid peroxidation may be involved in the pathogenesis of CAD through up-regulation of signaling pathways leading to tissue damage.

p-800
PURIFICATION OF NATURAL AND RECOMBINANT PROFILIN FROM PERSIAN MELON

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Profilin is an ubiquitous, low-molecular weight, actin-binding protein involved in the organization of the cytoskeleton of eukaryotes, including higher plants. Profilin has been identified as a panallergen in pollens and fruits. Poly-l-proline affinity chromatography is the most common method in purification of profilin. For various reasons, this method is not efficient enough to separate profilin from fruits. The aim of this study was evaluation of immunoaffinity chromatography for purification of natural profilin. In this study, recombinant Cuc m2 (rCuc m 2), has been cloned and expressed in E. coli and then purified with metal affinity chromatography. After expression of rCuc m2 protein, rabbits were immunized with this protein.Total IgG was purified from the sera of immunized rabbits with rCuc m2, using Protein-A column. Then purified IgG was bound to the CNBr activated Sepharose 4B column. Afterwards, melon extract was loaded on the column. In the next step, all the unwanted proteins were washed from the column. Separation of profilin from its specific IgG was done by means of Glycin-HCl buffer (pH, 2.8). Purified natural and recombinant profilins were analyzed by SDS-PAGE and Western blotting. SDS-PAGE analysis showed protein components, at the molecular weight range of profilin (14.5 KD, monomer or polymer). These protein bands showed immunoreactivity with IgE of patients sensitive to melon. Our findings show that immunoaffinity.

p-801
THE EFFECT OF GREEN TEA EXTRACT ON OXIDATIVE STRESS AND SPATIAL LEARNING IN STREPTOZOTOCIN-DIABETIC RATS

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Diabetes mellitus is associated with disturbance of cognitive functioning. Hyperglycemia induced oxidative stress has been proposed as a cause of memory complications of diabetes including cognitive impairment. In the present study, the effect of green tea extract (GTE), a potent free radical scavenger was investigated against spatial impairment in streptozotocin-diabetic rats. Eight weeks after diabetes induction, GTE (3mg/L) was administration in drinking water. The learning and memory behavior was evaluated and the rats were sacrificed for estimation of oxidative stress parameters. The green tea extract showed improve cognitive impairment in diabetic groups but these changes were not significant. There was also significant increase in total antioxidant capacity (FRAP), and the level of total thiol groups in green tea treated group vs control. This study demonstrated the effectiveness of GTE on spatial impairment and oxidative stress induced diabetes mellitus.

p-802
OXIDATIVE STRESS AND ANTIOXIDANT DEFENSE IN RELATION TO THE SEVERITY OF CORONARY ARTERY DISEASE

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Introduction: Oxidation of low-density lipoproteins (LDL) is believed to play an important role in atherogenesis, and antioxidant vitamins are thought to protect against coronary artery disease (CAD). Materials and Methods: We investigated if vitamin E and reduced glutathione concentrations in plasma are associated with the severity of CAD as assessed by a semiquantitative scoring system in which coronary angiograms are analyzed for the number and size of distinct stenotic lesions (global stenosis score). Plasma malondialdehyde (MDA) levels were measured as a marker of oxidative stress. The study group consisted of 65 consecutive patients with angiographically proven CAD aged<45 y and 55 age- matched control subjects. Results: Lipid-adjusted plasma vitamin E and glutathione concentrations were significantly lower in the patients than in control subjects (all P<0.05), whereas the absolute plasma vitamin E concentrations did not differ significantly. Plasma MDA concentration was significantly higher in patients compared with control subjects (P<0.005). No association was found between the plasma concentration of vitamin E and the stenosis score. In contrast, a significant inverse correlation were found between lipid-adjusted plasma values of vitamin E concentration and the global coronary stenosis score (r= -0.477, P<0.001) or plasma malondialdehyde concentration (r = -0.385, P< 0.01). An inverse correlation was found between vitamin E and glutathione with MDA (all P <0.05). Conclusions: We conclude that a low vitamin E, as antioxidant, might play a role in the development of stenosis in coronary arteries and may contribute to clinically manifested CAD.

p-803
BIOCHEMICAL AND HISTOLOGICAL STUDY OF THE PROTECTIVE EFFECT OF SODIUM TUNGSTATE ON OXIDATIVE STRESS INDUCED BY STREPTOZOTOCIN IN PANCREAS OF DIABETIC RATS

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Purpose: The aim of this study was to evaluate the effect of sodium tungstate on oxidative stress in pancreas of streptozotocin induced diabetic rats. Materials and Methods: Sixty mature Wistar male rats (Age: 2-3 month) were selected and divided into six groups randomly (n=10). Control (C), STZ-induced diabetic (D), STZ-induced diabetic rats treated with sodium tungstate in drinking water one week before induction of diabetes by STZ and throughout the experiment (TDB), food-restricted diabetic group (FRD) with an equal amount of food as that consumed by the TDB group, healthy control rats treated with sodium tungstate (TC), diabetic rats treated with sodium tungstate one week after STZ administration (TDA). Food and fluid intake of all groups were measured daily; and body weight, blood glucose and insulin were measured every week. At the end of the treatment period and after an overnight fast, all animals were sacrificed under light ether anesthesia. Immediately, blood samples were collected from tail vein. The pancreas was quickly removed and fixed in modified Lilli's solution. A portion of pancreas was placed in cold saline solution and used for the determination of antioxidant potential. After tissue blocking in paraffin, 4 μm thick sections were prepared and stained for granulated beta-cells by the modified aldehyde fuchsin method. Stained sections were examined by light microscope. Glucose levels were measured by oxidase-peroxidase enzyme method, and serum insulin levels were determined by ultra sensitive rat insulin kit, using ELISA. The lipid peroxidation product (MDA) was measured in nanomoles of MDA-TBA complex. The thiobarbituric acid reactive substances (TBRAS) were expressed per milligram of tissue protein. Antioxidant power of blood and pancreas were measured by ferric reducing/antioxidant power (FRAP). One-way analysis of variance (ANOVA) followed by Tukey´s post hoc test for multiple comparisons were used to compare differences between experimental groups. Significant level was set at $P< 0.05$. Results: Blood glucose levels of TDB group were lower than, D, TDA, and FRD groups ($P< 0.01$); and were comparable with control rats in the period of treatment. Blood insulin levels of TDB, TDA, D and FRD rats were lower than C and TC rats ($P < 0.01$). In addition, blood and pancreas antioxidant power were increased in TDB rats compared to D, TDA and FRD groups ($P< 0.01$). Likewise, blood and pancreas lipid peroxidation were decreased in DTB rats compared to D, TDA and FRD groups ($P< 0.01$). Histological study showed that granulated beta cells in TDB rats were greater than D, TDA and FRD groups. Conclusion: Results demonstrate that sodium tungstate can reduce oxidative stress and increase antioxidant power in rats. Thus, sodium tungstate treatment before STZ injection can preserve pancreatic beta cells from STZ-induced damages.

p-804

EFFECTS OF NEWLY SYNTHESIZED PREPARATIONS OF COUMARIN NATURE ON RAT BRAIN THROMBOPLASTIC ACTIVITY

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High level of activity and sensitivity to various effects of chemical and physical compounds is a specificity of mammal's brain thromboplastins (T). Our previous studies established phospholipid-dependence of thromboplastic activity (TA) in different tissue systems including erythrocytes. The present study is devoted to the investigation of the effects of a synthetic preparation of coumarin derivative (allyl thioureido-3-carboxycoumarin) coded in the present study as GSh-16. It is established that it has hepatic protective and also psychotherapeutic effects. We succeeded to study the dynamic changes of TA of the brain homogenate of white rats at different periods of time after intravenous and inter-abdominal introduction of the preparation. The results obtained demonstrated the anticoagulant effects of GSh-16 manifested as a statistically verified prolongation of prothrombin time (PT) for about 18% when used with T isolated from brain homogenate 10 min after introduction ls of 0.5 ml of 1% solution of the preparation to animals. The observed shift appears to be demonstrated more in 30 min after the introduction of the same dose of GSh-16 when PT is in the limits of 27%. The results show the time-dependence of the observed effect of the preparation. It is observed that prolonging of PT took place after the injection of 1 ml of the preparation in 10, and 30 min for 27% and 36% in comparison with the control, respectively. It is necessary to note that in spite of the observed increase of the inhibiting effect of the doubled concentration of GSh-16 on TA of brain tissue, the PT is not directly proportional to the quantity of the preparation used. This preparation is recommended as an effective anti-coagulant.

p-805

EFFECT OF ALTHAEA OFFICINALIS AND CITRUS BIGARDIA WATER EXTRACTS ON NITRIC OXIDE PRODUCTION IN CULTURED VASCULAR ENDOTHELIOMA CELLS

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Nitric oxide (NO) is produced by endothelial cells, neutrophils, macrophages and neurons. The reaction in which nitric oxide is synthesized is catalyzed by nitric oxide synthase (NOS). There are three isoforms of NOS: endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS). Released NO from these cells has physiologic and pathologic effects. In traditional medicine Althaea officinalis and Citrus bigardia flowers have been used for treatment of inflammation, headache and muscular spasm. In this study we measured the effect of water extract of these plants in concentrations of 0.2, 0.5, 1, 1.5, 2, 2.5 and 3 g/L on NO production. The water extract of Althaea officinalis and Citrus bigardia significantly reduced NO production at first in comparison to control ($p\leq0.05$). But by increasing the concentration of the water extract, NO production significantly increased and then decreased again. The increase by Citrus bigardia water extract was not significant. The results show that various concentrations of the water extract of these plants have different effects on NO production.

p-806
MOLECULAR ANALYSIS OF IRANIAN PATIENTS WITH DUCHENNE/BECKER MUSCULAR DYSTROPHY

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Duchenne muscular dystrophy (DMD) and the milder allelic Becker muscular dystrophy (BMD) are X-linked disorders. Both DMD & BMD result from heterogenous mutation in the dystrophin gene and in about 65% of the cases one or more exons of the gene are deleted or duplicated. One third of cases arise from new mutations and the rest are familial. To analyze the prevalence of deletion in Iranian patients, a deletion screening was performed on group 18 exons of dystrophin gene. Deletions were detected in 56% of patients. Seventy four perecent of deleted exons were located in the major hot spot region, whereas 26% were in the minor hot spot ones. The most frequently deleted exons were exons 50, 48 & 47 showing 16.2%, 16.2% &12% deletions, respectively. No deletion was detected in exon 43.The intragenic RFLP analysis (Pert87-15/BamH1 & Pert87-8/Taql) were carried out on DNA samples obtained from 22 Iranian unrelated families (196 males & females) showing DMD & BMD clinical symptoms, and 45% of them had informative patterns.The perecentage of heterozygosity was 22.75% for bamH1 intragenic RFLP, and 22.75% for taq1 intragenic RFLP.

O-807
ETHYL ACETATE EXTRACT OF TEUCRIUM POLIUM ATTENUATES CELLULAR DEOXYRIBOSE-INDUCED OXIDATIVE DAMAGE AND APOPTOSIS

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Reducing sugars are known to produce reactive oxygen species (ROS) mainly through the glycation reaction. 2-deoxy-D-ribose (dRib), a sugar with a large reducing capacity, has been known to induce oxidative stress and cell death in different cell lines. Teucrium polium is a medicinal plant used in folk medicine for various therapeutic purposes such as diabetes. In our pervious studies, we have demonstrated that Teucrium polium has the potential of reducing the blood glucose level as well as oxidative stress parameters in streptozotocin-treated diabetic animals. The effect of ethyl acetate (EtOAc) extract of Teucrium polium on dRib-induced oxidative damage and apoptosis in K562 cells was investigated. K562 cells exposed to dRib (50 mM) exhibited abnormal properties, including decreasing cell viability, overproduction of ROS, glutathione depletion and biochemical features of apoptosis. Lipid peroxidation was also increased in dRib-treated K562 cells. Treatment with EtOAc extract of Teucrium polium at different concentrations (5-50µg/ml) reduced ROS production, glutathione depletion and lipid peroxidation in K562 cells under dRib condition. Furthermore, EtOAc extract attenuated dRib-induced apoptotic markers such as DNA fragmentation and annexin-V staining. It can be concluded that EtOAc extract of Teucrium polium prevents ROS-induced apoptosis through antioxidant mechanisms.

p-808
ESTROGEN INDUCED APOPTOTIC PATHWAY IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Introduction: Systemic lupus erythematosus is an autoimmune disease which mostly occurs in women during their childbearing years. The strongest risk factor for development of SLE is female gender and hormones. Estrogen enhances immune responses and in SLE pathogenesis it is important to understand how nuclear antigens become immunogenic because, for development of the disease nuclear antigens have to be accessible to immune system. Apoptotic cells are potential reservoirs of altered auto antigens. In promoter region of some genes such as FasL there is an estrogen responsive element, which binds estrogen and induces apoptosis and apoptotic related molecules gene expression via activation of death receptor. In this study we intended to investigate the molecular mechanisms of the effects of estrogen on apoptotic related molecules on T lymphocytes from SLE patients at gene expression levels. Methods: Study group comprised of 35 SLE patients, and 20 age and sex matched controls. T lymphocytes were cultured with estrogen. For detecting gene expression, RNA was isolated from cells, cDNA synthesized and using specific primers the expression levels of Fas, FasL, Bcl-2, Caspase 8 and Caspase 9 were determined. Results and Conclusion: Estrogen induced Fas/FasL pathway of apoptosis in lupus patients is accompanied by significant enhancement of FasL and Caspase 8 genes expression and significant increased percent of apoptotic cells implying more susceptibility of patients to estrogen. There were no significant changes in expression levels of Fas, Bcl-2 and Caspase 9 genes.

p-809
THE POSSIBLE ROLE OF CRYOGLOBULINS IN THE PATHOGENESIS OF SCHIZOPHRENIA

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The immune system dysfunction, including alterations in mechanisms of both specific and non-specific immune response and development of autoimmune reactions, is involved in the etiopathogenesis of schizophrenia (Scz). However, the molecular pathomechanisms responsible for these alterations have not been studied well. This study aims to determine quantitative and qualitative characteristics of cryoglobulins (Cgs) in the blood of Scz-affected subjects. Cgs are abnormal immune complexes, where both antigen and antibody are presented by immunoglobulins. Cryoglobulinaemia is a nonspecific marker of the activation state of the immune system, inflammation and autoimmune

sensitization. Also Cgs may bind complement components and activate the complement that is a major effector system of innate immunity involved in the Scz-associated immune system alterations. Fifty-five schizophrenic patients and 30 age and sex matched healthy volunteers were involved in this study. Cgs were isolated by exposure of blood serum samples to precipitation at low temperature followed by extensive washings of Cg-enriched pellets. Total concentration of protein in Cgs was determined according to the method of Lowry et al. The immunochemical composition of Cgs was analyzed using different electrophoretic and immunoblotting systems. The results demonstrated elevated levels of type III (polyclonal IgG, IgM and IgA) Cgs in the blood of Scz-affected subjects. Western blot analysis of Cgs revealed the presence of C1q and C3 complement proteins and their activation products in Cgs isolated from the blood of Scz-affected subjects. In conclusion, we suggest that Cgs are responsible for the activation of complement system and development of autoimmune processes in Scz.

p-810
THE COMPLEMENT SYSTEM AND CIRCULATING IMMUNE COMPLEXES IN POST-TRAUMATIC STRESS DISORDER

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Post-Traumatic Stress Disorder (PTSD) is a psychiatric disorder frequently found in psychiatric clinic and in the populations of victims of traumatic events. PTSD, characterized by an intense fear, helplessness or horror, resulting from exposure to a traumatic event, is clinically manifested with three main syndromes: reexperiencing, avoidance behavior and numbing of emotion, and physiological hyper-arousal. PTSD is accompanied by a number of specific and non-specific "somatic" pathologies, such as cardiovascular, immune and physical complaints/chronic pain. The aim of this research was to study the important role of the immune reactions in the pathogenesis of PTSD. The detection of circulating immune complexes (CICs) and complement activation is essential for the understanding of disease pathomechanisms, especially from the point of view of immunity. For this reason we examined the total hemolytic activities of alternative and classical pathways of the complement and the level of CICs in the serum of 23 PTSD patients within 13 years from traumatic event and 27 healthy subjects. The results demonstrated a significant increase in the mean values of CICs concentrations and the total hemolytic activity of complement by classical pathway and a significant decrease in the complement alternative pathway of hemolytic activity in the serum of patients with PTSD in comparison to a healthy group. The data obtained clearly indicate the involvement of CICs and the classical pathway of complement activation in pathogenesis of PTSD. However, to draw final conclusion, further investigations are required.

p-811
THE COMPLEMENT SYSTEM IN FAMILIAL MEDITERRANEAN FEVER WITH AND WITHOUT COLCHICINE THERAPY

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Familial Mediterranean fever (FMF) is an inherited autoinflammatory disease occurring mainly in Mediterranean and Middle Eastern populations. FMF is characterized by periodic attacks of fever accompanied by serosal membrane inflammation. Colchicine has been the chosen drug in the treatment of FMF since 1972. Although the possibility of multiple immunologic mechanisms has been studied, the actual mechanism is still unresolved. The present study evaluates the role of inflammatory immune reactions in the pathogenesis of FMF and the influence of colchicine therapy on these reactions. As the indicators of the inflammatory response, the total hemolytic activities of alternative and classical pathways of the complement and the activities of the individual complement components C1, C4, C2 and C3, were determined in the serum of patients with FMF (n=19; with regular colchicine treatment and n=18; without colchicine treatment). In comparison to healthy subjects, significant increase of the total hemolytic activity of the complement and the hemolytic activities of complement components were detected in the serum of colchicine-free patients. But in the serum of patients receiving regular colchicine treatment the differences of these parameters were not significant. However, a decrease of the hemolytic activities of complement by alternative pathway was found in both cases. In summary, the inflammatory attacks and the response to colchicine therapy in FMF may be better explained upon studying the activation of a complement cascade. The relation between the ability of colchicine to suppress complement activation and its efficacy in FMF treatment requires further investigations.

p-812
HCG HYDROLYZING ANTIBODIES IN SERA OF HEALTHY PREGNANT WOMEN

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The first natural catalytic antibody, now termed abzyme, which hydrolyzes intestinal vasoactive peptide, was discovered by Paul et al. Various catalytic antibodies or abzymes have been detected recently in the sera of patients with several autoimmune pathologies e.g. systemic lupus erythematosus, and rheumatoid arthritis. During pregnancy and immediately after delivery women very often experience autoimmune processes similar to those in autoimmune disease

patients. Numerous antibodies have been discovered in the milk of healthy human mothers which are capable of hydrolyzing proteins, DNA, RNA or polysaccharides. hCG is an essential hormone in pregnancy which is produced mainly by syncytiotrophoblast cells of the preimplantation embryo and is considered to play an essential role in the establishment and maintenance of early pregnancy. In this study, IgG fractions were isolated from the sera of healthy pregnant woman by subsequent steps of chromatographic purification on protein G Sepharose, and Sephacryl S-300. The proteolytic activity of electrophoretically homogenous IgG antibodies was demonstrated by in gel assay with gelatin as substrate. Anti-hCG autoantibodies were shown to be present in the sera of most pregnant woman, as judged by ELISA results. These IgG fractions were incubated with hCG and it was shown that IgGs of these samples were able to hydrolyze hCG. We did not detect any protease activity on other substrates like HSA. Several abzymatic criteria were applied and it was shown that hydrolyzing activity belongs directly to antibodies not to any protease contamination. We demonstrated the proteolytic activity of polyclonal IgG antibodies from the blood of healthy pregnant women for the first time. Also our findings demonstrate the generation of polyclonal antibodies with hCG hydrolyzing activity by the immune system of healthy pregnant women.

p-813
CATALYTIC AUTOANTIBODIES AND DIABETES

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Diabetes Mellitus is a prevalent and chronic disease in modern societies. In traditional classification, this kind of diabetes is classified in two types: type 1(IDDM) and type 2 (NIDDM). In type 2 diabetes, it has been shown that autoantibodies are present in majority of patients. These auto antibodies react against auto antigens of pancreatic islet β cells such as GAD, IA-2 and IAA. This phenomenon indicates autoimmune character of this disease. In this study, IgG fractions were isolated from sera of 3 diabetic patients and 7 control subjects by affinity chromatography on protein G- Sepharose. Then the purity of these IgG antibodies was confirmed by SDS-PAGE and Western blot analysis. According to our data, in homogenous antibody preparations, there is no detectable protein contamination. The proteolytic activity of electrophoretically homogenous IgG antibodies was confirmed by zymogram analysis with gelatin as substrate. Then antibodies from 3 diabetic patients and 7 non diabetic control subjects were incubated with insulin, at 37° for 6 days in PBS. In diabetic patients, Insulin degradation was clearly observed by acetic acid- urea polyacrylamide gel electrophoresis. Our results may likely propose a new mechanism for the incidence of this disease.

p-814
DETERMINATION OF POTASSIUM BASE LINE IN VITREOUS HUMOR OF THE RIGHT AND LEFT EYES OF DEAD MEN AND WOMEN IN KHOOZESTAN

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Diuretics, cardiac glycosides, β blockers, angiotensin E inhibitors and NSAIDs increase potassium in cardiovascular patients. The rise of potassium concentration in extra cellular fluids (such as vitreous humor) will arrest the heart. For this reason, the measurement of potassium concentration in the extra cellular fluids such as vitreous humor which is anatomically isolated and deteriorates more slowly than other body fluids is necessary for diagnosis of metabolic disorders, electrolytes disorders, sudden death, infarction and investigation of crime in forensic medicine. During 2006 to 2007, the present study was done on 88 bodies (44 males, 44 females) brought for autopsy in forensic medicine of Khoozestan. The time of death was recorded by relatives or responsible coroners. From each eyes 2 ml of vitreous humor was aspirated by 2 ml syringe from outer canthus. Samples were frozen at -20^{o} C. The samples were then thawed at room temperature and each sample was centrifuged at 3000 rpm for 5 minutes and the supernatant fluid was taken for determination of potassium by flame photometry and values were expressed in mEq/L. Statistical analyses showed no significant differences when the samples were taken from both eyes at the same time and analyzed separately. It was also observed that there was no effect of gender on the levels of vitreous humor potassium. So potassium base line in vitreous humor in right and left eyes of dead men and women in Khoozestan ($Y = 1.03x + 3.94$, $r=1$) was determined.

p-815
COMPARISON OF COENZYME Q10 CONCENTRATIONS IN HL-60 LEUKEMIA CELLS AND NORMAL LYMPHOCYTES

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An increasing amount of experimental and epidemiological evidence implicates the involvement of oxygen derived radicals in the pathogenesis of cancer development. Ubiquinol is an endogenously synthesized lipid-soluble antioxidant. Regeneration of ubiquinol from the oxidized form is essential to the maintenance of its antioxidant function. Our goal was to determine the importance of Q10H2 as an antioxidant in cancer. The cultured cells of HL-60 and human normal lymphocytes were assayed in cell free systems obtained from specific number of both HL-60 and normal lymphocytes. The protein concentration was determined by Bradford method. Q10H2/ Q10 assayed by HPLC at absorbance 275nm and its ratio in HL-60 was2.4±1.7 and in normal lymphocytes

was1.09±0.433 .Our findings show that in HL-60 leukemia cells Q10H2/ Q10 concentration increases.

p-816
IMPACT OF KIDNEY TRANSPLANTATION ON CIRCULATING GLUTATHIONE PEROXIDASE ACTIVITY

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Accumulating data suggest that impairment of renal function is linked with an alteration of GPx activity. The present study was undertaken to explore: 1) the impact of kidney transplantation on GPx activity and 2) association between GPx activities and the levels of classical marker of renal function (i.e. creatinine; Cr) .Referred candidates of first kidney transplantation to Immam Khomaine Hospital, Urmia (n=23; Age range 40-60 years) were recruited. Blood samples were collected at -1, 0, 7, 14 and 21 days, respectively) and plasma obtained by centrifugation. GPx activity was determined spectrophotometrically. Data analyses were performed using SPSS software package (version 11). This study was approved by the ethical committee at UUMS. Mean GPx activity of pre-operation day (day -1), was 41 U/L. The respective values on post-operation days (i.e. 0, 7, 14 and 21) were 76, 159, 217 and 216 U/L, respectively. Significant inverse relationship was obtained between GPx activities and Cr levels ($r = - 0.439$, $P<0.05$). These findings imply that monitoring GPx activity, in conjugation with routine marker of renal function, in kidney recipients may provide an additional diagnostic tool in evaluating operation outcome during hospitalization period and after discharge.

p-817
THE EFFECT OF ARTEMISIA DRACUNCULUS LEAF EXTRACT ON THE ADHESIVE PROPERTY OF HUMAN PLATELETS

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Metastatic tumors are very common in the late stage of cancer. Platelet adhesion to the tumor cells plays a crucial role in the event of tumor metastases. The present study was undertaken to investigate the effect of the methanolic crude extract and chloroform fractions of Artemisia dracunculus leaves on platelet adhesion properties. Artemisia dracunculus (tarragon) is used as an anticoagulant and anticancer in Iranian folk medicine. Human platelets were prepared and incubated with different concentrations of the test sample (equivalent to 1-100 µg of plant leaf powder/mL) for different time intervals. The treated and untreated platelets were then activated with thrombin (0.25 U/mL) for 30 min and their adhesion to U937 cells was evaluated. Based on our observations, the methanol crude extract and its chloroform fraction at a concentration of 75 µg /mL, inhibited platelet adhesion to U937 cells by 61% and 77%, respectively. These data clearly indicate that affinity of platelets for U937 cells is reduced by methanol extract and fractions of A. dracunculus leaf. Thus, A. dracunculus is probably capable of mediating tumor metastasis through effecting cell adhesion property of the cells.

p-818
PRODUCTION AND PURIFICATION OF STREPTAVIDIN FROM STREPTOMYCES AVIDINII

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Streptavidin is a tetrameric protein produced by Streptomyces avidinii. It binds up to four molecules of a small water-soluble vitamin, D-biotin (vitamin H). The high affinity interaction of streptavidin/biotin (Kd=10-15 M) and the ability of streptavidin to withstand a wide range of pH, detergent and ionic strength conditions makes it an ideal system for many in vitro and in vivo applications. In this study the production of streptavidin from S. avidinii was examined in two synthetic media and one complex medium in several different growth conditions. Studies indicated that complex medium containing multiple carbon sources resulted in higher yields of streptavidin than synthetic media. Streptavidin was purified in a one step process from centrifuged fermentation broths by binding the protein to an iminobiotin Sepharose column at pH 11 followed by elution at pH 4.0. The purified protein was obtained in yields of 30 mg per liter of bacterial culture. Streptavidin purity and activity were checked by Western blot and ELISA analysis.

p-819
PRODUCTION AND PURIFICATION OF RABBIT ANTI-IGY ANTIBODY

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Anti-IgY antibody is an important tool in immunological laboratory for ELISA methods. In this study we prepared anti-IgY in two stages: 1) purification of IgY from chicken blood and 2) production and purification of anti-IgY from rabbit. Chicken serum fractionated by ammonium sulfate in final concentration of 50% and dialyzed against phosphate buffer (70mM, pH 8) to remove its salt content. Samples were loaded on DEAE-Sephadex ion exchanger. IgY rich fractions were eluted with phosphate buffer (70 mM, pH 8). Finally the purity of antibody was shown by SDS-PAGE. Then three rabbits were immunized against IgY by subcutaneous injection of an emulsion of 100µg of the IgY in 1ml of normal saline and an equal volume of complete Freund's adjuvant to rabbits. Booster injections were given intramuscularly using 50µg of the IgY in 1ml normal saline and 1ml of incomplete Freund's adjuvant in 15 day intervals. Then 10 days after the third booster, serum was collected for ELISA assay to indicate

rabbit immunization. Then, 100 ml of blood was collected and 20ml serum prepared. Serum IgG was precipitated with ammonium sulfate in a final concentration of 50% and dialyzed against phosphate buffer (25mM, pH 6.3). Samples were loaded on DEAE-Sephadex ion exchangerand anti-IgY rich fraction was eluted with phosphate buffer (25mM, pH 6.3). Finally the purity of antibody was shown by SDS-PAGE. Result of the affinity calculation on the basis of Beatty procedure was shown to be approximately1.39×109M-1. Activity calculation showed that anti-IgY has a good affinity for application in ELISA methods.

p-820
THE EFFECT OF DIFFERENT CHEMICAL TREATMENTS ON ELIMINATION OF CO(II) BY BROWN ALGA CYSTOSEIRA INDICA

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The heavy metal pollution in the industrial waste water is an ambient problem of worldwide interest. The majority of heavy metal salts are soluble in water and consequently cannot be separated by conventional physical processes. Marine alga, Cystoseira indica has shown impressive binding capacities for a wide range of heavy metals. In the present study, biosorption of Co(II) was performed by chemically treated Cystoseira indica in continuous system with residence time equal to 2.5 min at 25°C and pH = 4. Different chemicals ($MgCl_2$, Na_2CO_3, H_3PO_4 and formaldehyde) were used individually or collectively for treatment of intact alga. At the initial feed concentration of 72 ppm, the best result was obtained by the mixture of formaldehyde and H_3PO_4 with dynamic capacity (DC) of 40.1 mg/g of dry biomass and saturated capacity (SC) of 50.2 mg/g of dry biomass. The treatments with $MgCl_2$, Na_2CO_3, H_3PO_4 and formaldehyde showed DC to be equal to 15.9, 24.7, 29.3 and 22.0 with SC equal to 32.2, 39.5, 35.2 and 38.7 mg/g of dry biomass, respectively. In addition, the final swelled volume of formaldehyde treated alga increased to 38.2 ml while intact biosorbent swelled to 27.2 ml with relative standard deviation of 3.1 and 6.0 %, respectively. As a result, the treated biomass is a suitable alternative for conventional methods to eliminate heavy metals from aqueous solutions.

p-821
COMPARISON OF FREE LEPTIN TO TOTAL RATIO IN WOMEN WITH POLYCYSTIC OVARIAN SYNDROME AND NORMAL WOMEN AND THEIR CORRELATION WITH OBESITY

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Leptin has been implicated in the regulation of food intake, energy expenditure, and whole body energy balance in humans. Leptin is found in circulation in the free form and bound to receptors (sOB-R). PCOS (polycystic ovarian syndrome) is a common disorder characterized by menstrual dysfunction, obesity and oligo-anovulation. The known association between leptin and obesity suggest that leptin may have a role in PCOS. The purpose of this study was to evaluate the ratio of free to total leptin levels and their correlation with obesity in women with PCOS. Twenty seven women with PCOS and 27 normal women with matched BMI were studied Total leptin levels were measured by an ELISA kit. To separate free leptin, Sehadex G-100 gel filtration chromatography was used. After separation of free leptin, it was measured by an ELISA kit. The ratio of free to total leptin levels were significantly higher in women with POCS (p 0.044) and also were more in individuals with high BMI(BMI >25) (p 0.00) In conclusion, the mean ratio of free leptin to total leptin, similar to total leptin, is related to BMI and body fat.

p-822
DETECTION OF HUMORAL IMMUNOLOGIC FACTORS IN RENAL AND LIVER TARNSPLANTATION

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Background: Immunoglobulin abnormalities in organ transplant recipients have been described in literature, but data on graft recipients are still rare. Aim: Investigation of the pattern of immunoglobulin variation after human solid organ transplantation. Patients and Methods: This study included 18 liver transplant patients (18-52 years) and 14 renal transplant ones (21-42 years). Serum Immunoglobulins (IgG, IgA, IgM) were assayed by nephelometry before and at different periods after transplantation (one sample during 1-3 days and 2 samples during 1-4 months after transplantation). Results: In liver transplant patients serum immunoglobulins (IgG and IgA) increased in some patients before transplantation. A rapid drop in IgG, IgA (but not IgM) was observed during the first days after transplantation. Mild transient hypogammaglobulinemia (IgG) was present in five cases. Serum Immunoglobulins (particularly IgG and IgA) remained stable within normal or near normal limits during the following months after liver transplantation. In renal transplant patients serum immunoglobulins (IgG, IgA, and IgM) remained within normal or near normal limits during the first days, and during 1-4 months after transplantation in 13 of 14 cases. Hypogammaglobulinemia (IgG) was present in one of our renal transplant recipients. Conclusions: Very few studies have focused on time course changes of serum immunoglobulin concentrations after human solid organ transplantation. It may be interesting for several reasons: 1) A screening for hypogammaglobulinemia is useful after transplantation especially in patients with an intensified immunosuppression. 2) Immunoglobulin levels after transplantation may predict the risk of infections by capsulated microorganisms. 3) Serum immunoglobulin increase is a feature of chronic liver diseases, so the profile of serum immunoglobulin variation after liver transplant may add an insight into the pathogenesis of hypergammaglobulinemia of liver diseases.

p-823
RELATIONSHIP BETWEEN SERUM LIPID LEVELS AND THEIR OXIDISABILITY IN MYOCARDIAL INFARCTION

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Introduction: Acute phase response following myocardial infarction (MI) results in increased serum lipid oxidation susceptibility. Low density lipoprotein (LDL) oxidation plays a pivotal role in atherogenesis. Very few studies have investigated the relation of serum lipid peroxidation and lipid levels in MI patients. The aim of this study was to evaluate this relationship. Methods: Sixty four MI patients were randomly selected (23 females and 41 males, age 60 +/- 12 years). The level of serum lipids and lipoproteins were determined by the routine laboratory methods. The serum lipids oxidation was evaluated by monitoring conjugated diene after adding copper ion to the diluted sera. A number of quantitative parameters including lag-time, maximum speed rate of oxidation (V-max) and maximum amount of lipid peroxide products (OD-max) were then evaluated. Results: The levels of total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) were correlated with V max ($r = 0 .5$, $p < 0.001$ and $r = 0.53$, $p < 0.001$, respectively) and OD-max ($r = 0.32$, $p = 0.011$ and $r = 0.32$, $p = 0.01$, respectively). There was no significant correlation between lipid levels and other parameters of lipid peroxidation. Conclusion: It is suggested that in MI patients, total cholesterol and cholesterol-rich lipoproteins affect the final extent of in vitro serum lipids oxidation, while the extent of resistance to initiation of serum lipid oxidation is not affected by serum lipid levels in these patients.

p-824

CORRELATION BETWEEN PLASMA TOTAL HOMOCYSTEINE CONCENTRATION AND RISK OF THROMBOSIS

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Background: Elevated plasma concentration of the sulphur containing amino acid homocysteine (Hcy) is associated with increased risk of atherosclerosis and arterial thrombosis. The mechanism by which Hcy exerts these effects has yet to be fully elucidated, although a variety of possible mechanisms have been proposed, including endothelial dysfunction or haemostatic abnormalities. Aims: In this study, increase in homocysteine as an in depended risk factor for thrombosis and frequency of hyperhomocysteinemia was investigated in Iranian patients with thrombosis. Methods: In this study (case control) ,a total of 100 patients with arterial thrombosis (54 men , age 38± 13 years; and 46 women , age 35± 13 years) as case group and 68 healthy controls (40 men, age 36±10 years; and 28 women , age 35±13 years) were included in our study. Some information about patients such as age, sex, thrombosis history, and familial thrombosis history was also reported. We measured fasting plasma homocysteine in patients and control groups by ELISA method. The results were analyzed statistically by SPSS using t-test, chi-squares and also analyzed by odds ratios. RESULTS: A statistically remarkable difference was observed between the mean of fasting plasma total homocysteine in patients (XHcy=23.85 micro mol/lit) and the mean of plasma total homocysteine in the control (XHcy=11.48 micro mol/lit), ($P<0.001$).The frequency of hyperhomocysteinemia in patients was 48%, whereas this ratio in controls was 17.6% ($P<0.001$). A significant difference was obtained between the frequency of hyperhomocysteinemia in men (70.4%) and women (21.7%) in the patient group ($P<0.001$). There was moderate correlation between increase homocysteine and age in the patient group($r=0. 2$, $P=0.05$). In this study odds ratio was OR=2.72, 95% CI, 1.56-4.73. Conclusion: According to odds ratio (OR=2.72), hyperhomocysteinemia is an independent risk factor for thrombosis. For this reason, it seems that the measurement of homocysteine should be advised in patients with thrombosis or patients with a risk of thrombosis. Also elevated plasma homocysteine is associated with low serum Vit B12 and folate, and dietary supplementation with low doses of folate and Vit B12 should be considered for affected individuals.

p-825

IDENTIFICATION OF ASPERGILLUS SPECIES BASED ON MOLECULAR ANALYSIS

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The recent reports of reduced antifungal drug susceptibilities among some Aspergillus species strongly necessitate the rapid and accurate identification of Aspergilli to the species level. Furthermore, precise identification of Aspergilli is of great importance for epidemiological purposes and as a guide to clinical management. The conventional laboratory identification of Aspergillus species is mainly based on macroscopic and microscopic morphological characteristics, which have not only limitations in identifying atypical and non-culturable fungi but also they are practically time-consuming. Therefore, in this study, a molecular technique was set up for the accurate and efficient identification of aspergillus species causing numerous aspergillus infections. To date, different targets for the molecular identification of Aspergilli have been utilized including mitochondrial cytochrom b gene, a putative aflatoxin pathway regulatory gene (aflR), the DNA topoisomerase II gene (TOP2), and various rRNA gene regions. According to literature survey, the most promising target is the internal transcribed spacer 1 and 2 (ITS1 and ITS2). The reasons for this are the existence of 100 copies per genome along with their potential capability for precise and accurate detection of Aspergilli. ITS1 and ITS2 are located between the 18S and 28S rRNA genes. In our work, four known reference fungi were selected to evaluate the efficiency of using ITS region in addition to setting up our experiment. Universal primer for ITS region was designed based on bioinformatic analysis of data obtained from a few hundreds of Aspergillus species ITS regions by multi-alignment. To amplify ITS region, PCR was performed on the four extracted DNA from Aspergillus fungi using the designed primer. The resulting PCR products were sequenced and the data were blasted against NCBI database to identify the species of Aspergillus fungi. The blast results showed the accuracy of the procedure.

p-826
SOMATIC CHROMOSOME AND EVALUTION STUDIES IN SOME GENOTYPES OF HULL-LESS BARLEY

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Barley is a grass belonging to the family Poaceae, the tribe Triticeae, and the genus Hordeum (Nilan and Ullrich, 1993). About 90% of barley grain is used in alcoholic beverage production and as a livestock feed. Karyotyping studies were carried out on fiveteen hull-less barley (Hordeum vulgare L.) genotypes, using squash technique and aceto-orcein staining method. Chromosomal parameters examined were as follows: long arm (L), short arm (S), total chromosome length (TL), arm ratio (AR), r-value (S/L), form percentage of chromosome (%F), chromosome volume, relative length of chromosome (%RL) and the number of satellites. Genotypes tested were diploid (2n=2x=14). Satellite numbers differed, ranging from 1 to 2 pairs and differed in satellite length. The most chromatin length was detected in G9 (73.37 µm) while G15 demonstrated the least (30.85 µm). The type of chromosomes was determined as m in all genotypes, using Levan's chromosome nomenclature. Karyotypes were classified in 1A of Stebbin's classification. In addition to this, to test the karyotypic symmetry in more detail, other parameters, e.g. Romero-Zarco, total form percentage of karyotype (%TF), symmetry index (%S), coefficient of variation (%CV), and dispersion index (DI) were also considered. For instance, in Romero-Zarco method, the A1 and A2 coefficients were 0.37 (G2) and 0.46 (G9), respectively. The first 3 principal component analysis (PCA) justified 94% of the total variations Correlation was also determined for cytogenetic parameters. Cluster analysis was carried out for either chromosomal parameters classifying in 2 classes.

p-827
EXPLOITATION FROM MULTIVARIATE ANALYSIS TO OBTAIN CYTOGENETIC VARIATION AND EVOLUTION STUDIES IN SOME HULL-LESS BARLEY GENOTYPES

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The present paper describes the karyologic analysis in sixteen genotypes of hull-less barley for the first time. Karyotypic studies were carried out on hull-less barley (Hordeum vulgare L.) genotypes using squash technique and 2% (w/v) aceto-carmine staining method. Chromosomal parameters examined were as follows: long arm (L), short arm (S), total chromosome length (TL), arm ratio (AR), r-value (S/L), form percentage of chromosome (F%), Chromosome volume, total chromatin length (X) and the number of satellites. In chromosomal parameters, total chromosome length highly and significantly correlated positively with short arm. When cytogenetic correlation between number of traits are significant, it shows that we do not have high variation of genotyps from such characters. Principal component analysis of karyological data showed that the first three components possessed 94% variation. This was also important in observed variations. Cluster analysis of karyological data and ordination of taxa on the first two PCA axes are obtained. The cluster analyses make up the second major cluster, indicating second genotypes distinctness. Grouping obtained from ordination of taxa based on the first two PCA axes supports the clustering results. Among the species, moving from class 1A (symmetrical class), dispersion index indicating symmetry were as follows: G13, G12, G11, G8, G1, G14, G3, G15, G4, G2, 7, G5, G10, G6 and G9. The first 3 principal component analysis PCA for chromosomal parameters justified 94% of the total variations. In general, the results of this study proposed selective genotypes for crossing in plant breeding with the most homology in chromosomal variations. Cluster analysis was carried out for either chromosomal parameters classifying in 3 classes.

p-828
INHIBITION OF PHOSPHOLIPASE A2 (PLA2) BY POMEGRANATE FRUIT EXTRACT

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Snake bites are the major medical concern in Iran & India. However, there is very little evidence to enable us to understand how to prevent mortality and morbidity. Phospholipases A2 (PLA2s) are commonly found in snake venoms and have side effects in living organisms. PLA2s gain considerable attention because of their role in various pathological conditions such as inflammation & other toxic properties such as envenomation. Natural and artificial inhibitors act on PLA2s through different mechanisms. Current anti- inflammatory drugs can cause side effects such as ulcer and bleeding, so design of specific and natural PLA2 inhibitors is an important option for the production of safe anti inflammatory agents. The peel, yellow membrane and seed of pomegranate fruit were separated manually, powdered and the pulp portion was lyophilized. 10gm of each part of pomegranate was extracted in 20ml of acetone & water by stirring at cold conditions for 4 hours. The suspension was centrifuged and then filtered. The acetone extracts of different parts were pooled separately and concentrated under vacuum at 40°C. All aqueous extracts were lyophilized. .For PLA2 assay, an amount of protein was chosen such that 40-60% hydrolysis of substrate was obtained at 37°C for 60 min. Total phenolic compounds were determined by Folin- Ciocalteu assay. Indirect hemolytic activity showed that the lysis by venom PLA2 was 100%. The results showed that acetone extract of pomegranate peel had maximum phenolic compounds and maximum PLA inhibition. In vitro data

suggests that acetone extract of pomegranate peel can be used as an inhibitor of PLA2 enzyme which is involved in inflammation.

p-829
EFFECTS OF CLOFIBRATE, NICOTIC ACID AND THEIR COMBINATION ON THE SERUM CONCENTRATION OF LIPOPROTEINS, TRIGLYCERIDE SAND CHOLESTEROL IN CARDIOVASCULAR DISEASES

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Introduction: some studies show that the use of antihyperlipidemic drugs such as nicotinic acid, clofibrate and their combination reduces the risk of cardiovascular disease. These drugs also reduce atherogenic lipoproteins and increase HDL-cholesterol. Materials and Methods: 92 patients with hyperlipidemia aged 43-79 years suffering from cardiovascular diseases were investigated. 78 patients received clofibrate (1 g twice daily), 7 patients received nicotinic acid (with an increasing dose of up to 2-2.1 g daily) and 7 patients received a combination of clofibrate and nicotinic acid (1 g clofibrate twice daily plus increasing doses of nicotinic acid up to 2- 2.1 g daily). The serum concentration of total cholesterol, triglyceride, LDL-C and HDL-C were measured before treatment, and six and fifteen weeks after treatment, respectively. Results: treatment with clofibrate after six weeks, significantly ($p<0.001$) reduced the total cholesterol (13%), triglyceride (20%), and LDL-C (16%). This drug also significantly increased the HDL-C (23%). After 15 weeks of treatment with clofibrate, a further decrease in total cholesterol (25%), triglyceride (55%), LDL-C(27%) and a further increase in HDL-C(32%) were observed in this study. Treatment with nicotinic acid after 6 weeks significantly ($p<0.001$) reduced the total cholesterol (6%), triglyceride (11.5%), and LDL-C(6%). Niacin also significantly increased the HDL-C (14%). After 15 weeks treatment with nicotinic acid a further decrease in total cholesterol21%), triglycerides (32%), LDL-C(13.5%) and a further increase in HDL-C (31%) were also shown. Also in this study a combination therapy with clofibrate and nicotinic acid after 6 weeks significantly ($p<0.001$) reduced the total cholesterol (18%), triglyceride (19%), and LDL-C(29%) and further increased HDL-C. Conclusion: combination therapy (clofibrate + nicotinic acid) for patients with cardiovascular diseases is more effective than either drug used alone in reducing the levels of the atherogenic factors such as total serum cholesterol and LDL-cholesterol and in increasing the anti- atherogenic factor HDL-C.

p-830
PURIFICATION OF CYTOCHROME P4501A FROM 3-METHYLCHOLANTERENE - TREATED PERSIAN ACIPENSER, ACIPENSER PERSICUS

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The cytochrome P450 1A subfamily is responsive to environmental contaminants such as polycyclic aromatic hydrocarbons. Because of its inducibility, it has been used as important tool for assessment of pollutants in aquatic environments. Pretreatment of sturgeon fish (Acipenser persicus) with 3-methylcholanterene increased liver microsomal cytochrome P450 and 7-ethoxyresorufin O-dealkylation. 3-methylcholanteren treatment caused a 22-24 fold increase in 7-ethoxyresorufin O-deethylase (7-EROD) activity of Persian Acipenser liver microsomes. Cytochrome P4501A was purified from the liver microsomes of Persian Acipenser collected in Shahid Behshti center, Rasht. Purification of cytochrome P4501A1 involved anion exchange chromatography of Emulgen LS114 and cholate solubilized microsomes on DEAE-Sepharose fast flow columns. The major fraction, cytochrome P4501A was further purified by polyethylene glycol and gel filtration on Sephacryl S-200 column. Cytochrome P4501A1, purified 14-fold with a specific content of 7.64 nmoles P450 per mg protein, produced a single band on SDS-polyacrylamide gel electrophoresis having monomer molecular weight of 58±1KDa. The Persian Acipenser P450 hemoprotein showed an absorption maximum at 477nm in CO-difference spectrum and a strong immunoreactivity with Mab anti cod P4501A1.

p-831
EFFECT OF DEXAMETHASONE (DEX) ON BAX EXPRESSION PATTERN IN MOUSE TESTICULAR GERM CELLS

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Programmed cell death (apoptosis) is an important regulatory process in spermatogenesis. The molecular mechanisms of controlling apoptosis have not been explained properly. The glucocorticoid (GC) drugs are one of the most commonly prescribed and effective anti-inflammatory agents used for the treatment of many inflammatory disorders. Evidence indicates that GC agents induce apoptosis in testis by decreasing the testosterone level. This study has focused on the effects of Dexametasone (Dex), as a member of GCs, on expression of Bax protein in mouse testicular germ cells. Bax is a member of BCl2 family and an important proapoptotic protein that may involve in this process. Fourteen young adult male (6- 8 weeks) NMRI mice randomly divided in 2 groups. Dex group received 7 mg/kg per day for 7 days. Control group received only saline daily atn the same time. One day after the final injection, the mice were killed. Then the testes were fixed in formalin and embedded in paraffin for immunohistochemistry (IHC) studies. Antigen retrieval was applied by using citrate buffer. Then, sections were immuno-stained with monoclonal antibody against Bax. The ABC staining method was carried out. Primary antibody was omitted as negative control. Positive immunoreactivity in different sections was evaluated. Immunoreactivity was assessed in all specimens. All spermatogenic cells showed positive immunoreactivity of Bax

expression in Dex group. Immunoreactivity was in the cytoplasm of the spermatogenic cells. The spermatocytes and spermatids showed very strong Bax expression. It appears that GCs such as Dex could induce apoptosis through proapoptotic proteins such as Bax.

p-832

DECREASE IN CYTOCHROMES BIOSYNTHESIS CONCOMITANT WITH INCREASE IN CATALASE ACTIVITY IN SALMONELLA TYPHIMURIUM GROWN ON POLYETHYLENE OXIDE SORBITAN MONO-OLEATE

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Salmonella typhimurium, a gram negative, facultative aerobic bacterium belonging to the enterobacteriaceae family, is a common cause of food poisoning worldwide and also a major threat to animal health especially damaging for the poultry industry. Part of S. typhimurium constant threat stems from its flexibility with respect to substrates. In this work, the bacterium was shown to grow on polyethylene oxide sorbitan mono-oleate (PSM) as substrate and some of its physiological parameters were studied under these growth conditions. S. typhimurium was grown in minimum medium supplemented with 0.5%, 1% and 2% PSM. The cytochrome content of stationary phase cells was measured by recording the dithionite-reduced-minus-H2O2-oxidized difference absorption spectra of cell suspensions. The wavelength pairs for cytochromes were 560-580 nm for cytochrome b560 (extinction coefficient 14.8 mM-1 cm-1), 595-606.5 nm for cytochrome b595 (extinction coefficient 1.9 mM-1cm-1), 628-649 nm for cytochrome d (extinction coefficient 18.8 mM-1cm-1). Catalase activity was measured spectrophotometrically by monitoring at 240 nm the H2O2 consumption by various cell suspension aliquots. Results showed that cytochromes b595 and d were undetectable in cells grown on PSM while the cytochrome b560 content was reduced to about 10% of the control grown on glucose. On the other hand, the catalase content of cells grown on PSM increased to up to 25 times that in the control grown on glucose. Our results support the view that evolutionary catalase was present in aerobic organisms prior to the respiratory chain and that the existence of catalase suffices to support cell growth in an aerobic system.

p-833

SERUM LEVELS OF SIALIC ACID AND NEURAMINIDASE ACTIVITY IN CARDIOVASCULAR, DIABETIC AND RETINOPATHY PATIENTS

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Background: Sialic acid is a component of serum that is elevated in diseases such as diabetes and certain malignancies. The normal range of SSA concentration and serum neuraminidase activity in different populations are varied, probably due to racial differences. Objective: The purpose of the present study was to obtain the average SSA concentration and serum neuraminidase activity, in an Iranian population, and to show weather these indices could indicate the severity, and serve as risk factors ,for diabetes and CVD. Methods: Serum sialic acid (SSA) concentration and neuraminidase activity were measured in 214 male and female patients and 150 normal individuals. The patient groups were composed of diabetics, diabetics with vascular disease, CVD, diabetics with retinopathy and retinopathic patients. A mean ± SEM value of 60.06 ±3.36 mg/100 ml for SSA and50.82 ±2.93 mU/ml for serum neuraminidase activity were obtained for randomly selected normal controls. Results: SSA was significantly higher in the patient groups as compared to the values in the age and sex –matched controls. Increased SSA in the diabetics with vascular complications was significantly higher than that for diabetics with retinopathy. The serum neuraminidase activity was also increased in the patient groups. In contrast to the pattern for SSA levels, serum neuraminidase activity in the diabetic patients was not significantly lower than that for diabetics with retinopathy. Conclusion: while serum neuraminidase activity may serve as a factor which tends to increase in CVD, diabetic ,and retinopathic patients, it may not be as reliable as the SSA level which correlates with the severity or monitoring of these diseases .However, it can be a useful index to be used along with SSA measurement.

p-834

ABNORMAL CHROMATIN INTEGRITY MAY SERVE AS A SIGNAL FOR SPERM UBIQUITINATION

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Introduction: Ubiquitin, an 8.5kDa peptide, marks other proteins for proteasomal degradation, and tags defective sperm during epididymal passage. Thus sperm ubiquitination seems to be a universal marker for sperm defects. It is not known how such spermatozoa are recognized by epididymal ubiquitination pathway. In order to find the main trigger for sperm ubiquitination, we examined the relationships between sperm ubiquitination, sperm chromatin integrity and clinical sperm parameters. Methods: Semen samples from 63 couples attending Avecina infertility clinic, were collected and analyzed according to WHO criteria. Each sample was evaluated for sperm surface ubiquitination, by direct immunofluorescent method using FITC-labeled anti-ubiquitin antibodies. Chromatin integrity of the same semen samples were analyzed using Acridine orange and Toluidin blue staining test. Results: There was a positive and significant correlation between ubiquitinated sperm and the percentage of sperm with abnormal chromatin (AO: r = 0.58, p= 0.000 and TB: r =0.48, p=0.000). Negative correlations were obtained between sperm ubiquitination and: sperm count (r = -0.2, p=0.048, sperm morphology (r = - 0.36, p= 0.003), rapidly progressive motility (a) (r = - 0.25, p= 0.044) and slow progressive motility (b) (r = -0.28, p=0.022). Also sperm ubiquitination positively correlated to the percentage of immotile sperm (r= -0.38, p= 0.002). Discussion: A positive correlation obtained between sperm ubiquitination and sperm

chromatin integrity suggests that ubiquitin-dependent sperm quality control has the ability to detect sperms with abnormal chromatin integrity and target such ubiquitin-labeled sperms for epididymal degradation. However DNA integrity is not the only signal targeting sperms for ubiquitination.

O-835
THE STUDY OF GENE EXPRESSION AND ULTASTRUCTURAL CHARACTERISTICS IN THE DIFFERENTIATED ENDOTHELIAL CELLS FROM HUMAN BONE MARROW MESENCHYMAL STEM CELLS

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Angiogenesis is an essential biological process for embryonic development, organogenesis, wound healing, reproductive tissue cycles, and tumorigenesis. Human bone marrow mesenchymal stem cells (HBMSCs) can be differentiated into mesenchymal tissues like osteocytes, chondrocytes, and adipocytes in vivo and in vitro. The aim of our study was to investigate gene and protein expression of the endothelial cell specific markers and ultrastructural characteristics in the differentiated endothelial cells of capillary like network from HBMSCs which were generated out of mononuclear bone marrow cells from healthy donors separated by density gradient centrifugation. HBMSCs were confirmed for the specific markers by flowcytometric analysis. The differentiation of HBMSCs into endothelial cells was done by cultivation of confluent cells in the presence of vascular endothelial growth factor (VEGF). Immunocytochemistry, flowcytometry analysis, RT-PCR and transition electron microscopy (TEM) were used for the detection of the specific endothelial markers. The results showed that the isolated HBMSCs were positive for CD105, CD44, and CD166 markers and negative for CD34. Capillary network formation showed in vitro angiogenesis well. A strong protein and gene expression of the von Willberand factor (vWF) and vascular endothelial growth factor receptor-2 (VEGFR2) and CD31 was observed in the differentiated endothelial cells. TEM analysis of the differentiated endothelial cells showed the endothelial specific organelles such as Weibel-Palade bodies, Caveolae which were absent in the HBMSCs. In conclusion HBMSCs may be an alternative source for differentiation into the endothelial cells for clinical therapies like tissue replacement or vascularization of artificial organs. In addition, the in vitro differentiation of HBMSCs might be a useful model for the elucidation of the role of VEGF and the other factors for differentiation and maturation of endothelial cells and capillary network formation.

p-836
EFFECTS OF MORPHINE ON ANTERIOR LOBE OF PITUITARY GLAND IN MALE RATS

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Introduction: One of the difficulties of human societies is dependency to opioids such as morphine. The influence of morphine on pituitary secretion through μ receptors is nearly known. The aim of this research is to study the effects of morphine on adenohypophysis cell proliferation that has not been studied so far. Materials and Methods: This study has been carried out on 12 adult male Wistar rats that were in two groups of morphine dependent and independent. They were addicted through morphine consumption during 21 days. After controlling the withdrawal syndrome signs and anesthesizing, the blood samples were taken and prolactin test was done by ELISA method. After performing cardiac perfusion, the hypophysis of animals was removed and fixed in 10% formalin. After staining, the number of adenohypophysis cells in two groups was calculated and compared. Results: The mean of prolactin production in dependent group as compared to independent group showed significant difference. Cell counting and comparison using histochemistry method indicated no significant difference between acidophil, basophil or chromophobe cells in morphine dependent and independent groups. Conclusion: In previous studies, some paradoxical information was explained about the effect of morphine on adenohypophysis hormones. In this study, morphine could elevate blood prolactin significantly but it could not change the number of adenohypophysis cells substantially.

p-837
EVALUATION OF THE RELATIONSHIP BETWEEN THE CHEMICAL COMPOSITION OF KIDNEY STONES AND DIETARY RISK FACTORS

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The prevalence of kidney stone formation is significantly high in the population of the world and it continues to be a significant cause of patient injury. Avoiding the consumption of certain foods may be useful in preventing such disorders. The aim of this study is to find the relationship between the chemical composition of the kidney stones and the type of food taken. The kidney stones (400) were received from the patients referred to the Biochemistry department by Urologists. Stone analysis was done using reliable methods. A standard questionnaire was filled by each patient about the type and the amount of foods on the customary diet basis. The results showed a significant relationship between the animal-protein consumed and formation of uric acid as well as calcium oxalate stones. A relationship was observed between consuming oxalate containing foods and drinks including black tea, juice having vitamin C, green vegetables and

nephrolithiasis. There was a significant relationship between the urine volume and the stone formation. An association was found between consuming the soft drinks (Cola and non cola) and a history of stone formation. There was a strong relationship between the family history and stone formation. Therefore we conclude that, aberrations in diet and fluid consumption as environmental factors and also genetic factors are mostly the cause of kidney stone formation and, we suggest dietary modification to correct the situation to some degree.

p-838
HEAT SHOCK PROTEIN-27 (HSP27) ANTIBODY TITRES IN PATIENTS WITH CHEST PAIN

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Heat shock proteins (Hsps) are molecular chaperones, released in response to environmental stimuli, including oxidative stress. Previous studies have shown associations between antibody titres to several Hsps and cardiovascular disease (CVD), including Hsp-60, 65 and 70. We developed a sensitive ELISA method to measure Hsp27 antibody titres in serum, and applied this method to a group of patients with acute chest pain. Patients (n=61, 47% males; mean age 75±9 y; 15% with a family history of CHD; 52% hypertensive; 22% diabetic; 27% smokers; 40% dyslipidaemic by ATPIII criteria) were admitted for chest pain between June 2005 and July 2006. Blood was taken for blood tests including troponin I and lipid profile. Serum was also collected from control subjects (n = 64, mean age 50±10 y; 38% with a family history of CHD; 34% hypertensive; 3% diabetic; 31% smokers; 61% dyslipidaemic) who were hospital, or university employees. For the ELISA method , samples were run in triplicate using Hsp-27 coated and uncoated wells. Specific binding was determined by subtracting the mean absorbance of uncoated wells from the coated wells. Antibody titres were compared using ANOVA. Categorical data were compared using χ-square tests. The detection limit of the anti-Hsp assay was A450nm > 0.08 absorbancy units, with intra and inter assay CVs of approximately 5 and 10%, respectively. Antibody titres to Hsp27 were significantly higher in patients with chest pain (0.20±0.15) compared to controls (0.11±0.08, $p < 0.001$). However there was no significant difference between patients who were troponin I positive (0.17±0.15, n=27) versus those who were troponin I negative (0.22±0.15, n=34). Hence although antibody titres to Hsp27 were higher in patient with chest pain, this did not appear to be related to an acute coronary event.

O-839
MODIFICATION OF LYSINE RESIDUES IN LYSOZYME: EFFECTS ON AMYLOID FIBRILLATION

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Amyloid deposits are associated with chronic neuronal and systemic pathologies. They have fibrilar shapes and are formed of cross β-sheet structures, as the result of conformational change in the precursor protein. Many efforts are devoted to a better understanding of the fibril formation mechanism. In the present communication, acidic pH and high temperature were used to drive hen egg white lysozyme (HEWL) toward amyloid formation and the effect of chemical modification of lysine residues was observed on this process. A variety of techniques, ranging from transmission electron microscopy, ThT fluorescence , Congo red absorbance to far-UV CD were employed to characterize the fibrilization process. Chemical modification of lysine residues was performed by acetic anhydride and citraconic anhydride. While acetylation causes significant increase in the rate of fibrillization , citraconylation is ineffective. Structural characterization of HEWL with fluorescence spectroscopy and circular dichroism shows no considerable differences in the secondary and tertiary structures of the modified and native forms or the hydrophobic patches on their surfaces. Since acetylation results in neutralization of charges on lysine residues and citraconylation causes a conversion of charge (positive to negative), it could be suggested that an electrostatic factor may be involved in the observed effect. Using the hotspot prediction server "Aggrescan", residues 25-32 are predicted to be a probable critical hotspot region of HEWL. It is thus possible that modification of a lysine residue (K33) located in the same region is of importance in this connection.

p-840
THE COMPARISON OF PLASMA HOMOCYSTEINE AND C-REACTIVE PROTEIN LEVELS IN SMOKERS AND NON-SMOKERS

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Cigarette smoking has clearly been identified as an independent, major risk factor of cardiovascular disease. To compare the levels of two risk factors including homocysteine and C-reactive protein (CRP) in smokers and non-smokers, we investigated homocysteine and CRP plasma levels in 150 smoker men and 50 non-smoker men using ELISA kit. In smokers, plasma levels of homocysteine ($P<0.001$) and CRP ($P<0.001$) were all significantly higher than non-smokers. These results showed that the plasma homocysteine and CRP levels were increased in smokers and thus it is suggested that there maybe a relationship between the elevated two risk factors and increased risk of cardiovascular disease.

O-841
THE ROLE OF PHOSPHATE IN THERMAL AGGREGATION OF YEAST HEXOKINASE II

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Abnormal accumulations of filamentous protein aggregates are pathological hallmarks of many neurodegenerative diseases. The biochemical factors that induce protein aggregation are not clearly understood, and studying the specific role of additive molecules on this process are of interest. In this work, hexokinase was chosen due to its important role in the glucose metabolism. The thermal aggregation of yeast hexokinase II was investigated in the phosphate buffer and the extent of aggregation was measured by monitoring the increase in absorbance at 350 nm versus time. The optimal condition of enzyme aggregation was found to be phosphate buffer, 50 mM, pH=8 and 60°C. No aggregation was observed in phosphate concentrations lower than 35 mM. Changing the buffer to MOPS or HEPES buffer solution, at the same condition, the enzyme was not aggregated. On the other hand, adding 35 mM or more of phosphate ions to MOPS or HEPES buffer induced protein aggregation. The Tm of the enzyme was the same in all buffers used. Circular dichroism and florescence studies showed that the enzyme structure in the presence of phosphate ions is not affected, but kinetic measurement of enzyme activity revealed that the presence of phosphate produced a decrease in hexokinase activity. From these data, the type of inhibition was defined as competitive when the variable substrate was ATP. In conclusion, phosphate acts as inhibitor and induces aggregation in yeast hexokinase II.

p-842
SERUM URIC ACID IS ASSOCIATED WITH MICROALBUMINURIA IN PATIENTS WITH MYOCARDIAL INFARCTION

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Introduction: Microalbuminuria has been linked to cardiovascular (CV) risk in patients with diabetes or hypertension. Urinary albumin excretion rate also increases during myocardial infarction (MI). Serum uric acid (UA) is emerging as a novel risk factor for CV diseases. The aim of this study was to evaluate the relation of microalbuminuria to CV risk factors such as lipids and serum UA in patients with MI. Methods: The study population consisted of 64 patients with MI (age range, 37-85 years; 41 men, 23 women). Serum levels of total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglycerides (TG) and UA were determined by routine laboratory methods. Urinary albumin/creatinine ratio (ACR) was calculated as an index of urinary albumin excretion rate. Results: Urinary ACR was correlated with LDL-C ($r=0.30$, $p=0.045$), TC ($r=0.34$, $p=0.008$) and serum UA ($r=0.45$, $p<0.001$). Conclusions: The results of this study indicate that in patients with MI, correlation between urinary albumin/creatinine ratio and serum uric acid is stronger than correlation between urinary albumin/creatinine ratio and total cholesterol and low density lipoprotein cholesterol. Therefore, serum uric acid is associated with microalbuminuria in this patient group.

p-843
BIOCHEMICAL AND MOLECULAR STUDIES OF VITILIGO

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Vitiligo is an acquired pigmentary disorder of the skin of unknown etiology. Genetic factors, oxidative stress, and autoimmunity might contribute for precipitating the disease in vitiligo. Melanin synthesis starts with induction of POMC (pro-opiomelanocortin) that cleavage into small peptides such as α-MSH. α-MSH is an agonist of MC1R, a receptor that is located in melanocyte membrane. This protein is a member of the GPCR super family. Activation of MC1R by α-MSH hormone is positively coupled to the cAMP signaling pathway and leads to a stimulation of melanogenesis and activation of such key enzymes as a tyrosinase. In this study 18 vitiligo and 2 healthy control subjects were studied. Analysis of the MC1R and α-MSH genes performed with direct sequencing revealed that in MC1R gene 15 different missense mutations take place of which 7 were novel mutations and 8 were mutations that were reported previously as natural variants. One frameshift mutation caused by the insertion of one nucleotide was observed in one of the patients.However no changes were observed in α-MSH gene. Then we extracted tyrosinase from skin lesions and also from normal skin a patient and assayed its activity. We observed a lower tyrosinase activity in skin lesions compared to the normal skin.. Also we used the comet assay to evaluate DNA strand breaks in peripheral blood cells and observed that level of oxidative DNA damage in peripheral blood cells was increased in Vitiligo. Finally we used RIA test to measured α-MSH level in peripheral blood and we observed no change in its level in vitiligo compared to normal subjects.

p-844
EXTRACTION AND PURIFICATION OF L-ASPARAGINASE FROM E. COLI B

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L-Asparaginase is considered as an important antineoplastic drug in the treatment of acute lymphoblastic leukemia (ALL) and non-Hodgkins lymphoma (NHL). Isolation and purification steps include: crude extract preparation of E. coli B grown in TSB at 37ºC until late log phase. The cells were harvested by centrifugation followed by sonication. There are two asperginases in E. coli B designated as EC-I and EC-II. Only EC-II has antilymphoma activity. For elimination of the EC-I, crude extract was heated to 55ºC. Proteins of the resulting solution was precipitated with ammonium sulphate . After dialysis of the precipitate against Tris –HCl buffer for

further purification of the enzyme. The dialysed sample was applied to a Ssephadex G-150 column, followed by DEAE-Cellulose and CM-Cellulose chromatography. Molecular weight of the enzyme was determined by SDS-PAGE against markers with known molecular weight. The specific activity of the L-asparaginase was 62 U/mg of protein, and the yield of the enzyme was 26%.The fold of purification was 115 and the molecular weight was 65 KDa. Its purity was shown by electrophoresis in polyacrylamide gel.

p-845
STUDY OF ALTERATIONS IN ANTIOXIDANT ENZYMES IN CALLI OF ASTRAGALUS SPECIES UNDER SALINITY

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In plants, H2O2 and other ROS such as superoxide radicals (O2-), singlet oxygen (1O2) and hydroxyl radical (OH0) are produced under stressful conditions. Uncontrolled production of ROS can cause cellular damages, so plants protect themselves from these damages by using antioxidant enzymes such as SOD, POX and PPO to scavenge the ROS. Salinity is a major environmental stress in plant agriculture worldwide that adversely affects plant growth and metabolism. Astragalus L. is the largest genus of flowering plants containing up to 3000 species and has important medicinal properties. In this study, we determined the activity of some antioxidant enzymes and protein content in the calli of three endemic Astragalus species (A.squarrosus, A.strictifolius, A.vegetus) under different concentrations of NaCl (0, 50, 100, 150, 200 mM). The enzyme activity and protein content were determined by spectrophotometric method and isoforms of some antioxidant enzymes were characterized by PAGE and the SDS-PAGE system used for proteins. In some species, the activity of POX increased significantly. Also some changes in other enzymes were seen.

p-846
RELATIONSHIP BETWEEN PLASMA CHOLESTEROL, VON WILLEBRAND FACTOR CONCENTRATION, EXTENT OF ATHEROSCLEROSIS AND ANTIBODY TITRES TO HEAT SHOCK PROTEINS-60, -65 AND -70 IN CHOLESTEROL-FED RABBITS

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Epidemiological studies have shown an association between atherosclerosis, heat shock protein (Hsp) expression, and Hsp antibody titres.We aimed to investigate the time course of appearance of Hsp-60,-65 and -70 antibodies in the cholesterol-fed rabbit and to relate antibody titres to serum concentrations of von Willebrand factor (vWF),a marker of endothelial injury.Rabbits were fed with 0.25–1.0% cholesterol diet for 13 weeks. Plasma levels of anti Hsp-60,-65 and -70 IgG titres,were measured using in-house enzyme-linked immunosorbent assays (ELISAs) together with plasma vWF concentrations. Plasma titres of anti-Hsp-60,-65 and -70 antibodies were all significantly increased by weeks 5, 7 and 9 following commencement of the experimental diet compared with baseline ($P < 0.05$ for all). In non-cholesterol-fed rabbits, plasma levels of anti-Hsp titres were unchanged over this period.Increased plasma vWF concentrations were also found in the cholesterol-fed rabbits, reaching a maximum at approximately week 8, and falling thereafter. Furthermore, plasma vWF concentrations at 13 weeks correlated strongly with antibody titres to all three Hsps ($r = 0.90$, $P = 0.002$; $r = 0.80$, $P = 0.017$; $r = 0.86$, $P = 0.006$ for Hsp 60, -65 and -70, respectively) and titres were also strongly correlated with final plasma cholesterol concentrations in cholesterol fed animals ($r = 0.95$, $P = 0.002$; $r = 0.8$, $P = 0.001$; $r = 0.84$, $P = 0.01$, respectively). In cholesterol-fed rabbits, antibody titres to Hsp-60,-65 and -70 appear to rise in association with a marker of endothelial injury, peaking at approximately the same time (8 weeks) after starting a high cholesterol diet.

p-847
MOLECULAR ISOLATION AND PURIFICATION OF HUMAN ROTAVIRUS FROM FECES OF CHILDREN WITH ROTAVIRUS INFECTION

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Rotaviruses are the most common cause of severe gastroenteritis worldwide. These viruses possess segmented double-stranded RNA genome and a protein shell composed of three capsid layers: VP7 and VP4 in the outer, VP6 in the middle and VP1, VP2 and VP3 in the inner layer. The life cycle of rotavirus is also unique. Upon infection, RNA-dependent RNA polymerases associated with virus particles are activated, resulting in genome transcription and extrusion of the eleven viral mRNAs from such particles. The mRNAs not only direct protein synthesis, but also serve as templates for minus-stranded synthesis to yield dsRNAs. In this study, dsRNAs were extracted from children feces with rotavirus infection. BSC-1 cells were transiently transfected with the dsRNAs and after 72 h the transfected cells which showed strong CPEs were collected and used as viral seed to infect BSC-1 cells. After two successive infections, these cells were prepared for electron microscopy. The TEM results revealed complete triple- and double-layered viruses in cytoplasm of the infected cells. In conclusion, the dsRNAs could amplify and synthesize the viral proteins. Since there are no RNA-dependent RNA polymerases in BSC-1 cells, the sense strand of genomic segmented dsRNA of rotaviruses could also be transcribed by the cellular DNA-dependent RNA polymerases.

p-848
PROTECTIVE ROLE OF MELATONIN IN SPINAL CORD RADIATION MYELOPATHY

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It is well known that free radicals produced by gamma radiation act as a destructive agent in the spinal cord. Melatonin, a hormone with a proven antioxidative efficacy, crossess all morphophysiological barriers, including the blood brain barrier and distributes throughout the cell.The aim of this study was to assess the biochemical and histopathological effects of gamma irradiation and protection by melatonin on the spinal cord of rat. Four groups of rats were investigated: control group with no radiation and treatment; irradiation group receiving intraperitoneal injection of normal saline; melatonin plus irradiation group receiviing 100 mg/kg melatonin by i.p injection and melatonin group receiving injection of 100 mg/kg melatonin. The spinal cords of both irradiation groups were irradiated by 22 Gy of gamma radiation of cobalt system after 30 minutes of treatment with either normal saline or melatonin.The animals were killed 72 h after irradiation for analysis of malondialdehyde(MDA) , glutathione(GSH) levels and histopathological (necrosis, vascular density) study. The MDA levels in the irradiation group were significantly higher than control ($p<0.001$), but the GSH levels were significantly lower ($p<0.01$). Administration of melatonin markedly reduced MDA ($p<0.001$) and increased GSH ($p<0.05$) levels in irradiated tissue. Overall histopathological changes were decreased in melatonin plus irradiation group in comparison to irradiated group. The data support the idea that spinal cord could be protected by using melatonin after irradiation.

p-849
F-BOX PROTEIN FAMILY MEMBER AT1G77000 IS RESPONSIBLE FOR A DRAMATIC MORPHOLOGICAL PHENOTYPE IN ARABIDOPSIS

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In the process of generating transgenic Arabidopsis lines, a randomly occurring mutant with a dramatic morphological phenotype was isolated. This line was named “bushy” according to the dramatically reduced plant size. Microscopic observations revealed defects on the cellular organization of leaves, trichomes and flower organs. In order to understand the molecular basis of the "bushy" mutant, T-DNA flanking sequences were isolated showing that the T-DNA was inserted in the promoter of the At1g77000 locus. At1g77000 encodes an experimentally so far uncharacterized F-box protein. F-box proteins regulate diverse cellular processes including cell cycle transition, transcriptional regulation and signal transduction. F-box proteins are part of SCF (Skp1p, cullin, F-box protein) complexes which mediate protein degradation by the proteasome. The T-DNA integration resulted in reduction of transcript levels of At1g77000. For complementation of the “bushy” phenotype and to study gain-of-gene function, the CDS of At1g77000 was fused with GFP, c-myc and HA-tags and will be transformed into homozygous “bushy” lines. To assess the transcriptional activity of the At1g77000 gene throughout plant development a 4.2 kb region upstream of the ATG was fused to the GUS reporter gene. Results from these experiments will be presented.

p-850
CHARACTERIZATION OF MONOCLONAL ANTIBODY AGAINST HUMAN SERUM ALBUMIN

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Hybridomas secreting monoclonal antibodies (MAbs) producing stable, specific and high affinity against human serum albumin (HSA) has been established. The aim of the present study is producing MAbs that will be potentially used in designing immunoassay methods especially immunochromatography assay kit for screening of microalbuminuria (MAU) in the early detection of diabetic and non diabetic nephropathy. The hybridomas were obtained by fusion of spleen cells from immunized mice with mouse myeloma cell line (SP2). After limiting dilutions three clones producing antibodies were designed as EMRC1-3 which displayed different pattern of fine specificity for HSA and low cross-reaction with other proteins as elucidated by inhibition enzyme-linked immunosorbent assay (ELISA). These clones were found to be of IgG class with k light chain. Subclass determination showed that all three MAbs secreted IgG1 type of antibody. The results of affinity purification for the two selected clones (EMRC1 and EMRC3) displayed high affinity with no cross reactivity with any of the related protein molecules. The stable hybridomas secreting anti-HSA were expanded in 50 ml flasks for large-scale production of the required antibodies. The standard curves were constructed with a sensitivity of 10 pg/well covering upto 100 ng/well. The high binding activity to HSA antigen and having no cross reactivity with other related molecules illustrated the potential application of these antibodies as an immunodiagnostic reagent in designing an immunochromatography assay kit for screening of MAU in diabetic and non diabetic patients.

p-851
PREPARATION, IDENTIFICATION AND CHARACTERIZATION OF MONOCLONAL ANTIBODY AGAINST MORPHINE

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A monoclonal antibody (MAb) was generated by immunization of Balb/c mice with conjugated morphine C6-hemisuccinated derivative (M-C6-HS) to cationized bovine serum albumin (cBSA).After three months the spleen cells of immunized mice were fused with myeloma cell of SP2/0 origin using 50% v/v polyethylene glycol (PEG). Supernatant of hybridoma cells was examined by ELISA, using M-C6-HS-BSA. Among several clones against morphine one of them was the best. This hybridoma was found to be of IgG2b class and contained lambda light chain.The antibody was purified by protein A affinity chromatography and the affinity of the MAb to morphine was 2.8×10^9 M^{-1} by non competitive enzyme immunoassay.The titer of supernatant of cell culture medium was at least 1/800. Finally the cross reaction of monoclonal antibody with some structurally related molecules such as codein and apomorphine was determined.

p-852
LEISHMANIA MAJOR HEAT SHOCK PROTEIN 70 (HSP70) STIMULATES STRONG HUMORAL RESPONSES IN CUTANEOUS AND VISCERAL LEISHMANIASIS PATIENTS BUT NOT PROTECTIVE IN MICE MODEL OF EXPREMENTAL CUTANEOUS LEISHMANIASIS

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Heat shock proteins (HSP) are highly conserved molecules involved in essential cellular functions, as well as in several immunological processes. In this study, we first immunized susceptible BALB/c and resistant C57BL/6 mice with the complete open-reading frame of Leishmania HSP70 (pcDNA-HSP70) and boosted mice with rHSP70 (amino acids 221-604 cloned in pQE-HSP70 and referred to as rHSP70) mixed with Montanide 720. When we evaluated the effects of HSP70 in both mouse strains, we found that the entire fragment (amino acids 221-604) and rCT-HSP70 (amino acids 491-604 cloned in pQE-CT), but not rNT-HSP70 (amino acids 221-291 cloned pQE-NT), contained the highest immunogenicity. However, after infectious challenge with Leishmania major, no efficient protective responses were observed in either mouse strain. The humoral immune responses against the different truncated forms of HSP70 suggested a mixed TH1/TH2 response in vivo. We then assessed infected susceptible and resistant mice for lymphoproliferative and cytokine responses against the truncated forms of HSP70. At 9-week post-infection, we observed no differences in those responses between vaccinated and control mice. Next, we initiated comparative studies in human patient samples, finding no significant proliferation against all three truncated forms of HSP70 in the cellular immune responses of 16 cured cutaneous leishmaniasis patients and 5 normal individuals. Sera from active cutaneous and visceral leishmaniasis patients, however, were reactive to all three forms of HSP70. This study demonstrates the potential of HSP70 in stimulating humoral responses in human and mice, so it could be selected as serodiagnosis tool. (Vaccine25, 2007, 4159-4169).

p-853
CIGARETTE SMOKING AND THE RISK OF MALE INFERTILITY

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Cigarette smoke includes a number of substances that have negative effects on male reproductive system and cause male infertility. In this research, we investigated the effect of cigarette smoking on sperm parameters in fertile and infertile men (n=200). Semen analysis was performed according to the World Health Organization guidelines to obtain volume, pH, vitality, sperm concentration, motility, morphology and seminal plasma fructose concentration (World Health Organization, 2000). Sperm concentration was determined with a Neubauer® counting chamber. Motility was expressed as the percentage of motile spermatozoa into 4 categories (immotile, flagella, slow and rapid). Morphology was determined according to the WHO criteria using the Papanicolaou's staining procedure. At least 300 cells were examined at a final magnification of 1000 ×. Our research showed that sperm parameters quality in smoker men was lower than nonsmoker men ($p<0.01$). Therefore, it appears that cigarette smoking is associated with reduced sperm quality and idiopathic male infertility in smoker men.

p-854
THE EFFECT OF TNF-α AND HEAT SHOCK ON TELOMERASE ACTIVITY IN K562 HUMAN MYELOID LEUKEMIC CELLS

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Telomerase is an enzyme that adds telomere repeat sequences to the 3´ end of DNA strands in leading strand, DNA polymerase is able to complete the synthesis of the lagging strand. Telomerase activity is observed in 80-90% of all cancer types that cause immortality in these cells. The biologists are interested to study the effect of natural and chemical agents which inhibit telomerase activity in order to inhibit the growth of cancer cells. Many reports indicate that, heat shock as a physical agent induces apoptosis and differentiation in leukemic cells. TNF- α is a multifunctional cytokine that causes cytotoxicity, differentiation, fragmentation of DNA. In this work we have studied the effect of mild heat shock and TNF-α on the activity of telomerase in human myeloid leukemia cell lines analyzed separately. Cells were treated with 0, 10, 100, 1000, 10000 and 100000pg/ml of TNF-α for 96 hr. Then the cells were collected and counted and assayed for proliferation with MTT assay and BrdU cell proliferation assay .Telomerase activity was measured with TRAP-PCR ELISA method. Our results indicated that telomerase activity and cell proliferation in heat shock treated cells decreased with time and temperature. Enzyme inhibition was about 30% as compared with control cells at 44 ,43 and

42 ºC at 15, 30 and 60 minutes ,respectively. Certain heat shock proteins seem to be expressed on the surface of malignant cells after hyperthermia and these proteins can act as growth factors. TNF-α does not have detectable effect on proliferation and telomerase activity of K562 cells shows resistance to this cytokine.

p-855
ANTIATHEROGENIC AND ANTIOXIDANT EFFECTS OF L-ILE IN CORONARY ARTERIES OF HYPERCHOLESTEROLEMIC ANIMALS

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Peroxidation of blood lipoproteins is regarded as a key event in the development of atherosclerosis. Some evidences suggest that oxidative modification of amino acids in LDL particles converts them to an atherogenic form which is taken up by macrophages. Therefore the reduction in oxidative modification of lipoproteins by increasing the plasma antioxidant capacity (AC) can be an effective method to prevent cardiovascular disease. It has been shown that some amino acids and polyamines have antioxidant activity. In this project antioxidant and anti fatty streaks effects of L-Ile have been investigated in hypercholesterolemic rabbits. Rabbits were divided into three groups as follows: negative control (normal diet), positive control (hypercholesterolemic diet) and treatment group (hypercholesterolemic diet + L-Ile in drinking water). Animals were fed for five weeks and then blood samples were obtained to analyse plasma cholesterol. TG, HDL, LDL, antioxidant capacity (AC), MDA, and conjugated dienes (CDS). Right and left coronary arteries were obtained for histological studies. No significant difference was observed in plasma CDS levels between treatment and hypercholesterolemic control groups ($P > 0.05$). The levels of plasma choesteroll, LDL, MDA, AC, HDL, and TG in treatment group showed significant changes in comparison with the positive control ($P< 0.05$). The mean size of produced fatty streaks also showed significant reduction in treatment group compared to positive control ($P < 0.05$). The results showed that L-Ile has antioxidant and antifatty streaks effects without any influence on plasma lipid levels in hypercholesterolemic rabbits.

p-856
THE EFFECT OF VITAMIN E ON ANTIOXIDANT ENZYMES AND LIPID PEROXIDATION IN SMALL INTESTINE OF STRPTOZOCIN DIABETIC RATS

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Introduction: Gastrointestinal morphological and functional disorders are frequent in patients and animals with diabetes. It is proposed that oxidative stress, a factor implicated in the pathogenesis of diabetic complications, contributes towards some of these disorders. This study investigates the effect of vitamin E on oxidative stress status of diabetic rat small intestine. Methods and materials: Twenty four male Wistar rats were divided into three groups; Control, nontreated diabetic (NTD) and Vit E treated diabetic (VETD).The increases in lipid peroxidation, protein oxidation, superoxide dismutase (SOD) and catalase activities of small intestinal mucosa in all groups were compared after 6 weeks. Results: There was no significant difference in catalase activity between NTD and control rats. The treatment with vit E significantly decreased lipid peroxidation and protein oxidation and increased catalase and SOD activity compared to NTD. Conclusion: The results revealed the occurrence of oxidative stress in the small intestine of rats during diabetes. Vitamin E as an antioxidant, attenuates lipid peroxidation and protein oxidation,and increases antioxidant defense mechanism.

p-857
EVALUATION OF THE ANTI-ULCER EFFECT OF ECHINOPS PERSICUS IN RATS

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Extract of Echinops persicus is traditionally used in Iran for treatment of cough and constipation. This extract is produced by the activity of a bug (Situphilus spp.) on this plant. We documented its anti-tussive effect in rats in our previous study. In this study we evaluate its anti -ulcer effect by Shay's method in rats. In 3 groups of rats, pylorus was ligated under anesthesia. The rats were euthanized after 19 hours later; and number and area of ulcer in stomach was measured. In group 2, extract was orally administered 45 minuets before pylorus ligation. In group 3, it was interapritoneally administered 20 minutes before pylorus ligation. The number of ulcers in stomach was significantly decreased in group 2 ($p=0.01$) and 3 ($p=0.037$) in comparison with group 1. The area of ulcer was significantly decreased in group 2 ($p=0.047$) compared with group 1. Thus, Echinops persicus may have anti-ulcer effect.

p-858
AN IN VITRO COMPARATIVE STUDY OF ASCORBIC ACID AND FSH EFFECTS ON THE MATURATION OF SYRIAN MICE FOLLICLES

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To investigate the combined and comparative effects of FSH and ascorbic acid on the in vitro maturation of mouse follicles and enclosed oocytes, intact preantral follicles were isolated from the ovaries of 6 week-old female Syrian mice and cultured in TCM-199 medium..10, 25, 50 and 100 IU/l concentrations of FSH and/ or 10, 50, 100, 200, 300 and 400 nmol/ml ascorbic acid were added to experimental plates containing 25-30 follicle enclosed oocytes. FSH concentration of 100 IU/l showed increased follicle diameter, survival, germinal vesicle breakdown (GVBD) and oocyte maturation rates ($P < 0.0001$). Ascorbic acid showed increased (59%) survival rate ($P < 0.0001$) but unaffected diameter, GVBD and oocyte maturation rates ($P > 0.05$) while, ascorbic acid and FSH containing medium showed a marked increase in all the parameters ($P < 0.05$) but follicle diameter was unaffected ($P > 0.05$). It is concluded that FSH and ascorbic acid increase the maturation rate of follicles and enclosed oocytes and if supplied in combination, growth rate increase significantly.

p-859
EPIDEMIOLOGICAL SURVEY OF SCORPION STINGING IN PATIENTS REFFERED TO SINA HOSPITAL, AHWAZ, IRAN DURING 2006-2007

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There are several reports in relation to scorpion stinging and its damage every year. Scorpion stinging is prevalent in Iran specially in six southern provinces and notably in Khouzestan province. The species is usually Hemiscorpius lepturus, a yellow type of scorpion. The venom of dangerous scorpions is neurotoxic and hemolytic. The most important clinical symptoms of scorpion bite are deep necrotic wounds, ankylosis, temporary or permanent psychosis, hemolysis and kidney failure. The aim of this study was an epidemiological survey of scorpion stinging in 759 patients reffered to Sina hospital, Ahwaz, Iran during 2006 – 2007. Biochemical and hematological tests such as hemoglobinuria, bilirubinemia, PT and PTT tests were performed in afflicted individuals. Among 759 stinged patients, 334 (44%) were men and 425 (56%) were women. Most of the stings were among age range of 15-22 years with 219 cases and the lowest number of stings was in the age group of > 62 . Although scorpion sting occurs in all seasons during the year, however not considering the species of scorpions, it had the highest prevelance in May – June with 173 cases and the lowest prevelance in December-January with one case. Scorpions were usually active during night and most of the stinging occurred from 7 PM to 6 AM at night. Considering the species of scorpions, it was observed that most stings occurred by the yellow type with 361 cases (47.5%), the black type with 182 cases (23.9%), and other types with 216 cases (28.4%). Our results indicated trace to +3 hemoglobinuria in 377 cases and bilirubinemia was <1/7 mg/dl. The results of PT and PTT tests were normal in 90% of patients. In spite of high mortality rates of scorpion stinging in Khouzestan province, specially by the yellow type such as Hemiscorpious lepturus, our study indicated no mortality due to early attention by patients and administration of specific anti-sera by nurses.

p-860
PROTECTIVE EFFECT OF VISCUM ALBUM LEAF EXTRACT AGAINST INDUCTION OF DIABETES BY ALLOXAN IN RAT

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Diabetes mellitus type I is due to synergistic effects of genetic, environmental and immonological factors. These factors can damage β-cells of pancreas through reactive oxygen species (ROS) and cause hyperglycemia.Viscum album leaves were reported to possess potent antihyperglycaemic and antioxidant activity and hence provide significant protection against oxidative stress.The purpose of this investigation was to examine whether viscum album leaf extract plays protective role against hyperglycemia and β-cells damages in alloxan treated rats. Viscum album leaves were extracted with hot water by stirring for 4h and evaporated to dryness under reduced pressure. Totally 90 rats were used and divided into three groups: 1) control rats 2) treated rats with high doses of Viscum album (1 gr/kg/day) 3) treated rats with low doses of Viscum album (0.5 gr/kg/day). Rats in groups 2 & 3 were pretreated with Viscum album extracts via gavage. Then alloxan was injected subcutaneously. After 24, 48, and 72h of alloxan injection, the animals were sacrificed by decapitation and blood collected and serum glucose by a commercial kit, serum insulin levels (by an ELISA kit) and serum total antioxidant power (by FRAP assay) were measured.Our results revealed that oral administration of viscum album leaf extract at both doses significantly inhibited the increase of serum glucose by alloxan ($p<0.05$). Also high and low doses caused a significant increase in serum insulin levels in pretreated rats after 24, 48, and 72h ($p<0.05$). Serum total antioxidant levels increased significautly in rats used high doses of Viscum album extract for 24, 48, and 72 h ($p<0.05$), while the difference was not significant in rats using low doses for 24h ($p<0.05$). Our findings suggested that Viscum album leaf extract are effective in reducing the oxidative damage to pancreatic tissue in experimental models of diabetes mellitus.

p-861
EFFECTS OF ARTIFICIAL CHAPERONE ON REFOLDING OF DENATURED α-AMYLASE IN THE PRESENCE OF METAL IONS

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Experimental refolding of the denatured proteins has been an important issue at the fundamental as well as at the biotechnological level. Recently a novel method of protein refolding, called artificial chaperone-assisted refolding, by sequential introduction of two low molecular weight agents, promotes the assembly of proteins native conformation. In the present study, refolding of GuHCL-denatured α-amylase was investigated using cationic detergent (CTAB) as the capturing reagent, along with β-CD and polymer solution containing metal ions (MgCl2,CaCl2 ,Ca(NO3)2 ,ZnCl2) as the stripping agents. Highest reactivation yield and lowest aggregation were observed upon addition of 8 equivalents β-CD and CaCl2 solution to α-amylase-CTAB complex. To improve the refolding yield and to suppress the extent of aggregation, the initial rate of the stripping step was slowed down by maintaining the refolding environment at 4 ^{o} C for about 3 min followed by raising the temperature to room temperature. Also, evaluation of the intrinsic fluorescence emission spectra of the refolded samples at the above-mentioned condition provides further supporting data regarding the higher native refolding yields. These data clearly indicate that CaCl2 had the highest efficiency among the metal ions evaluated for improving the artificial chaperone-assisted refolding of α-amylase.

P-862
RECOGNITION AND DETERMINATION OF HEPATITIS B VIRUS GENOTYPES IN KHUZESTANIAN PATIENTS BY MULTIPLEX NESTED PCR

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Background: Every year, between 10 and 30 million people worldwide are infected with the hepatitis B virus (HBV). More than 350 million people worldwide are chronically infected with HBV. On the average, 3% of people are carrier of HBV in Iran, but prevalence rate varies in different provinces. Eight genotypes (A-H) of HBV are known with variation in nucleotide sequence greater than 8%. It is believed that clinical course and outcome of antiviral therapy depended on genotype of the infecting HBV strain. Methods: In this study, HBV genotypes were determined by Multiplex Nested PCR with 6 pairs of HBV genotype-specific primers (A to F) in serum specimens from 100 hepatitis B patients of Khuzestan province. Results: Genotype D was detected in 66 of 100 (66%) patients, genotype B was detected in 22 of 100 (22%) while no product was obtained in 12 (12%) patients. Conclusion: our study indicates that the predominant genotype of hepatitis B in southwestern of Iran like other Mediterranean areas is genotype D.

p-863
IN VITRO ANTIOXIDANT AND FREE RADICAL SCAVENGING PROPERTIES OF VANADIUM-SALEN

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Reactive oxygen species (ROS) such as hydrogen peroxide (H2O2), superoxide anion (O2-), and hydroxyl radicals (OH•) have been implicated in degenerative or pathological processes. There is a balance between the generation of ROS and inactivation of ROS by antioxidant systems in oxygen-consuming organisms. Oxidative stress results from an imbalance between the generation of ROS and inactivation of ROS by antioxidant systems and plays a significant pathological role in human diseases. Vanadauim-salen has been proposed to act an antioxidant. Therefore, in the present study the antioxidant potency of this compound was investigated, employing various established in vitro systems such as free radical scavenging and inhibitory effect on protein oxidation as well as the inhibition of lipid peroxidation in rat liver homogenates. Results of this study revealed that this compound is a strong scavenger of (H2O2), (O2•-), and (OH•). The concentration of 1 μg/ml of this compound showed 99.6 % inhibition of lipid peroxidation in rat liver homogenates. Protein oxidation was inhibited by this compound in a dose-dependent manner. Based on our observations, it can be concluded that the new salen possess a good antioxidant capacity and therefore, further work is required to disclose its full antioxidant and pharmaceutical potential.

p-864
PREVALENCE OF SUBCLINICAL KETOSIS IN SUPER-POPULATION OF ALL HERDS IN TEHRAN REGION, USING SERUM BETA-HYDROXY BUTYRATE MEASUREMENT

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An arbitrary cutoff point of serum beta-hydroxybutyrate (BHB) is commonly considered as a perfect gold standard for the diagnosis of subclinical ketosis (SCK) which may lead to biased estimate of prevalence. The purpose of this study was to estimate the accuracies of serum BHB and glucose tests for the diagnosis of SCK and to estimate the true prevalence (distribution) of SCK in Tehran region. From 4 dairy farms around Tehran, 102 animals, at the third week of post parturition, were randomly selected and subjected to serum BHB and glucose tests, where cutoff point of > 1200 μm/L and < 35 mg/dL, respectively, were considered as positive test results. Bayesian methods were used to model the sensitivity (Se) and specificity (Sp) of the tests and the mean of the distribution of prevalences of SCK in super-population of all herds in Tehran region. Serum BHB was not found to be a perfect test for detection of SCK although it was highly accurate (with Se and Se of 0.96 and 0.93, respectively). Glucose had a low accuracy for monitoring or diagnosis of SCK (with Se and Se of 0.56 and 0.60, respectively). No conditional correlation was found between sensitivity and specificity of serum BHB and glucose at the selected cutoff points. The mean of the prevalence distribution of SCK for herds around Tehran was estimated to be 20.6%.Serum BHB

is not a perfect gold standard, and its accuracy should be considered when used in new diagnostic tests validation or prevalence estimation studies.

p-865
ASSOCIATION OF APOA-I GENE POLYMORPHISMS AND LIPID PROFILE AND TYPE 2 DIABETES IN AN IRANIAN POPULATION

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Dislipidemia is a common and complex metabolic disease with strong genetic basis. There are many candidate genes for phenotyping related to dislipidemia. ApoA-1 is the main protein in HDL particles. Dislipidemia appears with increasing blood levels of the cholesterol, triglycerides, chylomicron, LDL, VLDL or decreasing HDL. The correlation between two polymorphisms of apoA-I gene (G-75A, C+83T) and HDL level showed contraversial results in various populations. This study was carried out to illustrate the role of these polymorphisms in an Iranian population. A total 215 volunteers aged 25 to 75 was randomly selected based on the 17 area of Tehran municipality population in a case-control study. DNA extraction was done from their complete blood samples. Two specific primers were used for determining the alleles of these polymorphisms by PCR and RFLP. In the non diabetic population with dyslipidemia, we have found a significant relation between AA polymorphism and increasing of TG (P Value = 0.03). Also, there was a relation between GA polymorphism and increasing of uric acid (P Value = 0.045). There was no statistically significant relation between apoA-I gene promoter polymorphism and type 2 diabetes. In the same polymorphism, GA/AA allele has a protective role for dyslipidemia (P Value = 0.028). In C+83T polymorphism, the genotype CT is a risk factor for developing diabetes (P Value = 0.028). This study shows that apoA-I gene can be considered as the main goal for further etiologic studies and dyslipidemia treatment.

p-866
RELATIONSHIP BETWEEN OXIDATIVE STRESS AND SPERM DNA DAMAGE IN MEN WITH NORMOZOOSPERMIA AND ABNORMAL SPERM PARAMETERS

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Introduction: Oxidative stress and decrease in chromatin packaging quality has been shown to affect the integrity of the sperm chromatin and to cause sperm DNA damage. So, the aim of this study was to assess if the oxidative stress and amount of sperm nuclear protamine are related to sperm DNA damage in men with normozoospermia and abnormal sperm parameters. Materials & Methods: The study was done on 133 men who had been referred to Avesina Infertility Clinic, including 81 men which had abnormal sperm parameters and 52 normozoospermics. Semen analysis was done according to WHO criteria. Total antioxidant capacity (TAC/oxidative Stress index) in seminal plasma was measured by Randox kit using a spectrophotometric method. DNA damage was measured using toluidine blue (TB) test. Amount of sperm nuclear protamine was measured indirectly using Chromomycin A3 (CMA3) assay. Data were collected and analyzed using SPSS v.13. Results: sperm DNA damage and percentage of CMA3 positive sperms between the two groups showed a significant difference ($P<0.001$) but TAC showed no significant difference ($P = 0.329$). In addition there was no significant relationship between TAC and sperm DNA damage ($r = -0.172$, $P = 0.061$), but the relationship between sperm DNA damage and percentage of CMA3 positive sperms was significant ($r = 0.648$, $P< 0.001$). Conclusion: Sperm DNA damage was significantly higher in men with abnormal sperm parameters. One of its causes might be oxidative stress. In this study there was no significant difference in oxidative stress level between the two groups; however, due to protamine deficiency in men with abnormal sperm parameters, sperm DNA is not protected against ROS and oxidants can cause increasing fragmentation of the sperm DNA.

p-867
THE COMPARISON BETWEEN ELECTROPHORETIC PATTERNS OF EXTRACTED LPS FROM BRUCELLA ABORTUS BY PHENOL AND BUTANOL METHOD

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Introduction: LPS is one of the major components of the outer mambrane of gram negative bacteria and structurally and functionally is important. LPS is the dominant antigen at the surface of smooth strain of Brucella and is the major virulence factor. The LPS of Brucella has endotoxin activity but its activity differs from the LPS of entrobacteriaceae. Materials and Methods: Brucella abortus S99 was prepared from the collection of standard bacteria of Pasteur institute of Iran and cultured in a fermenter under controlled conditions and finally the cell biomass of Brucella was obtained. The LPS of Brucella was extracted through two different methods: 1.Phenol extraction (Westphal and Jann) 2.Butanol extraction (Morrison and Lieve). LPS was extracted from the aqueous and phenolic phases.The LPS content was assayed by Nowotny method and then the protein content of the extracted LPS was measured by Bradford method. Electrophoresis was used to detect and differentiate the protein bands the extracted samples. Results: Chemical analysis of two different extracted

LPS shows that the percentage of exracted LPS in phenol procedure and in butanol method is 1.1% and .0.9%, respectively. Protein content was measured by Bradford method. The concentration of the aqueous and the phenolic extracted LPS by phenol method was assayed separately.The protein content of aquaus and phenolic phase was 2.1% and 1.7%, respectively.The analyzis of electrophoretic movment of extracted LPS by Westphal and Morrison in the polyacrilamide gel countaining SDS slowed different patterns.

O-868
SERUM TRYPSIN INHIBITORY CAPACITY (TIC) AND ADENOSINE DEAMINASE ACTIVITIY IN PATIENTS WITH PSORIASIS

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Psoriasis is a chronic inflammatory skin disease characterized by pathological skin lesions due to various exogenous and endogenous factors and associated with a number of biochemical and immunological disturbances. The study was performed in a control group (n = 46) and in 40 patients with psoriasis. The patients were scored with PASI (psoriasis area and severity index). The serum levels of adenosine deaminase (ADA) activity was determined using Aguisti and Galanti method and trypsin inhibitory capacity (TIC) was measured by enzymatic assay. The serum levels of ADA of the psoriatic patients with active lesions were found to be significantly higher than the levels of the healthy control ($p<0.001$). We also found that the trypsin inhibitory capacity was significantly higher in patients than control group ($p<0.001$). Psoriasis is a chronic inflammatory skin disorder, the level of alpha 1 antitrypsin increased because this protein is an acute phase reactant protein. In the other hand adenosine deaminase (ADA) activity is a nonspecific marker of T cell activation. T cell activation is thought to play an important role in the pathogenesis of psoriasis.

p-869
COMPARISON OF TOTAL PLASMA HOMOCYSTEINE LEVELS IN PATIENTS WITH CORONARY ARTERY DISEASE (CAD) AND HEALTHY CONTROLS

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Background: Homocysteine is an amino acid with a free thiol (sulphydryl) group. Elevated plasma homocysteine concentration is a major risk factor for atherosclerosis and cardiovascular disease. Elevations in plasma homocysteine are typically caused either by genetic defects in the enzymes involved in homocysteine metabolism or by nutritional deficiencies in vitamin cofactors. Due to a high incidence of cardiovascular disease in our region and country, investigation about cardiovascular disease risk factors is very important. The purpose of this study was to compare total plasma homocysteine levels in patients with CAD and a group of healthy controls. Materials and methods: this study is a case-control study. The study group consisted of 50 patients with coronary artery disease (27 male and 23 females). The control group consisted of 50 healthy subjects (25 male and 25 females). Total plasma homocysteine concentration was measured by HPLC method. We also measured lipids and lipoproteins as known risk factors for cardiovascular disease. Results: The mean of total plasma homocysteine values in patients with coronary heart disease (20.59 µmol/l) was significantly higher than control group (12.78 µmol/l) ($p=0.001$).There were no significant correlation between total plasma homocysteine and other risk factors. Conclusion: Total plasma homocysteine is an independent risk factor for CAD in our study population. Since, the mean of total plasma homocysteine values in our patients and control group were relatively high in comparison to most other populations, it may be the main cause of a high rate of cardiovascular disease in some areas of Iran.

p-870
DEVELOPMENT OF A BIOSENSOR BASED ON INHIBITIORY EFFECT OF PARAOXON ON CHOLINE OXIDASE AVTIVITY

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Choline Oxidase (ChOx) is a flavin-dependent enzyme that catalyzes the oxidation of choline to betaine aldehyde through two sequential hydride-transfer steps. The study of this enzyme is important to the understanding of glycine betaine biosynthesis found in pathogenic bacteria or in economically relevant crop plants as a response to temperature and salt stress in adverse environments. In this report, effect of paraoxon (POX) on the activity of free and immobilized ChOx was studied. The results showed that POX has an inhibitory effect on ChOx activity. The inhibition was dependent on POX concentration. So that, by using an electrode coupled with immobilized ChOx, POX measurements can be carried out by determination of the decrease of substrate steady-state current. Quantitative evaluation of POX was done by electrochemical detection of H2O2 produced upon oxidation of the substrate (choline) at -0.05 V versus the internal screen-printed Ag pseudo-reference electrode. Some parameters which were effective on the biosensor performance were optimized. Based on this approach, using immobilized ChOx on screen printed electrode, POX can be detected with a detection limit of 2×10^{-7} M and an incubation time of 5 min.

p-871
INVESTIGATION OF HP0217 GENE IN H.PYLORI CAGA-POSITIVE AND NEGATIVE STRAINS ISOLATED FROM TALEGHANI HOSPITAL IN TEHRAN

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Frequent recombination of H. pylori differs between individual hosts, as well as between isolates from different continents. HP0217 gene plays a role in LPS biosynthesis and undergoes phase variation. This study investigated the presence of HP0217 and sequence analysis of PCR products in H. pylori isolates. 122 H. pylori isolates with positive PCR results for glmM were selected for analysis of cagA empty-site. After DNA extracting by Qiagene kit, the empty-site primers, which were derived from HP0216 and HP0218 two flanking genes, were used for PCR. Analysis of products predicted a yield of 1.5-kb amplicon, if HP0217 was present and a 0.5-kb if HP0217 was absent. Among 122 isolates cagA were detected in 89 isolates and both flanking genes were present in 40 of 122 isolates (with or without HP0217). HP0217 was detected in 8 of 40 isolates and 6 of them were cagA-positive. 32 of 89 cagA-positive and all of cagA-negative strains yielded a 555-bp amplicon. Sequence analysis of this amplicon confirmed the absence of HP0217 and revealed the presence of a 180-bp segment. HP0217 is one of the plasticity regions of H pylori with low G/C content which is strongly associated with adaptation to different environmental conditions. Although some studies were reported that HP0217 is found in H. pylori cagA-positive more frequently than in cagA-negative, but our findings showed that there was no significant correlation between HP0217 and cagA status. In addition the rate of HP0217 in Iranian isolates is lower. In as much as the primers were derived from HP0218/HP0216, lack of PCR product can not always be related to lack of HP0217. So more analysis should be done.

p-872
DEVELOPMENT OF RADIOMETAL-DOTA-ANTICD20 IMMUNOCONJUGATES FOR TARGETED THERAPY

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Rituximab was successively labeled with [64Cu]-copper acetate. The macrocyclic bifunctional chelating agent, N-succinimidyl-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA-NHS) was prepared at25^{o} C using DOTA, N-hydroxy succinimide (NHS) in CH_2 Cl_2. DOTA-Rituximab was obtained by the addition of 1 ml of a pharmaceutical solution of Rituximab (5 mg/ml, in phosphate buffer, pH=7.8) to a glass tube pre-coated with DOTA-NHS (0.01-0.1 mg) at 25^{o} C with continuous mild stirring for 15 h. Radiolabeling was performed at 37^{o} C in 3h. Radio-thin layer chromatography showed an overall radiochemical purity of 90-95% at optimized conditions (specific activity =30 GBq/mg, labeling efficacy; 82%). The final isotonic 64Cu-DOTA-rituximab complex was checked by gel electrophoresis for radiolysis. Radio-TLC was performed to ensure that only one species was present after filtration through a 0.22 µm filter. Preliminary biodistribution studies in normal rat model were performed to determine the complex distribution of the radioimmunoconjugate up to 28h.

p-873
THE EFFECT OF SULFUR MUSTARD ON GLUTATHIONE LEVEL AND ACTIVATION OF GLUTATHIONE-RELATED ENZYMES IN LUNGS OF RATS

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Sulfur Mustard (SM) is a potent alkylating agent with electrophilic properties, which reacts with such cellular macromolecules as DNA, RNA, and protein. It has been used as a chemical warfare agent. SM increases the formation of reactive oxygen species (ROS) such as superoxide anions, hydrogen peroxide, hydroxyl radicals, and lipid peroxides. Reduced glutathione (GSH) is an important intracellular antioxidant that reacts with oxygen free radicals and acts as a substrate for other detoxification enzymes such as glutathione peroxidase (GPX) and glutathione S-transferase (GST). Glutathione reductase (GR) requires NADPH for its activity, resulting in the reduction of oxidized glutathione (GSSG) to GSH. In this study, Wistar rats (150-250 g body wt.) were randomly divided into nine groups as follows: group 1 control and group 2 sham that received DMSO (dimethyl sulfoxide used as solvent), group 3-9 experimental groups that received SM (1-80 mg/kg) by intraperitoneal injection only once. 6 rats in each group were killed after 1, 2, 7 and 14 days of exposure. The lungs of each group were removed and the enzyme activities of GPX, GR and GST as well as GSH level were determined by biochemical methods. The results show that at concentrations lower than 10 mg/kg SM, the activities of GPX and GST were enhanced and no change in GSH level was observed, but at concentrations higher than 10 mg/kg, antioxidant enzyme activities were decreased as compared to the control group and the GSH level was significantly decreased. The data suggest that at concentrations lower than 10 mg/kg, the enhanced activity of antioxidant enzymes and no change in GSH level may be a compensatory response to scavenge reactive oxygen species generated by SM, while at higher SM concentrations (>10 mg/kg), SM induces oxidative stress by depleting the antioxidant defense systems.

p-874
THE EFFECT OF SULFUR MUSTARD ON ACTIVATION OF ANTIOXIDANT ENZYMES AND MALONDIALDEHYDE LEVEL IN LIVER OF RATS

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Sulfur mustard (SM) is an alkylating and blistering agent that readily reacts with a wide range of cellular macromolecules. Also, it can increase the rate of free radical formation in organs of the body. Superoxide dismutase (SOD) inactivates the superoxide radical. Catalase (CAT) inactivates hydrogen peroxide (H2O2). Malondialdehyde (MDA) is the end product

of lipid peroxidation. In this study, Wistar rats (150-250 g body wt.) were randomly divided into nine groups as follows: group 1 control and group 2 sham that received DMSO (dimethyl sulfoxide used as solvent), group 3-9 experimental groups that received SM (1-80 mg/kg) by intraperitoneal injection only once. 6 rats in each group were killed after 6, 24 and 48 hours, and 7 and 14 days of exposure. The livers of each group were removed and the enzyme activities of SOD and CAT as well as MDA concentrations were determined by spectrophotometric assays. The results show that at concentrations lower than 10 mg/kg SM, the activities of SOD and CAT were enhanced and no change in MDA level was observed, but at concentrations higher than 10 mg/kg, antioxidant enzyme activities decreased as compared to the control group and the MDA level was significantly increased.. The data suggest that at concentrations lower than 10 mg/kg, the enhanced activity of antioxidant enzymes and no change in MDA level may be a compensatory response to scavenge reactive oxygen species generated by SM, while at higher SM concentrations (>10 mg/kg), SM increased lipid peroxidation and oxidative inactivation of enzyme proteins and induced oxidative stress and cell death.

p-875
RELATIONSHIP AMONG ACUTE PHASE PROTEINS (HAPTOGLOBIN AND FIBRINOGEN), ALBUMIN AND CLINICAL FINDINGS IN DAIRY CALF PNEUMONIA

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Enzootic calf bronchopneumonia is a multifactorial disease that occurs in association with the interaction of various infectious agents, and calf suceptibility.The economic losses is associated with death loss and treatment costs, reduction of live weight gain and productive life span. Today, the most efficient disease control strategies are based on improvment of management, vaccination and treatment. Treatment is an integral part of any control program of respiratory disease in calves. The aim of treatment is to eradicate the pathogen and to relieve the adverse effects of the disease such as inflammation; pyrexia and depression.Treatment must be instigated as early as possible to reduce the possibility of long term pulmonary damage and the development of chronic pneumonia. The aim of this study was to examine the acute phase response in calves during enzootic pneumonia. We measured acute phase proteins (APP) and identified some potential markers useful for evaluation of calves' health status. Sixty Holstein calves were selected in this study. Clinical findings were recorded from physical examination of the animals. Two blood samples were taken from the calves, one having EDTA anticoagulant. Blood samples were used for CBC and serum biochemical analysis. The rsults of this study showed a significant increase in Hp and Fb. Our results indicated the application of haptoglobin and fibrinogen measurments as indicators of health in calf herds, thereby facilititating treatment decisions.

p-876
EVALUATION OF THYROID AUTO ANTIBODIES AMONG HYPO- AND HYPER- THYROID PATIENTS REFFERED TO AHWAZ GOLESTAN HOSPITAL IN 2005

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Introduction: Autoimmunity has variable frequencies among both hypo-and hyperthyroid diseases in different areas. There has been no study in this field in Ahwaz, Iran. This study was conducted to estimate anti- thyroid antibodies seroprevalence in hypo-and hyperthyroid patients, and to show the distribution of these auto-antibodies by age, sex and ethnicity. Materiasl & Methods: This was a cross-sectional study. During a period of 12 months, sera of 130 thyroid patients (46 hypo- & 84 hyper- thyroid cases) aged 2-70 years, 18 males and 112 females, from Golestan Endocrinology Center in Ahwaz, south west of Iran, and 100 sera from healthy subjects were collected. These sera were examined for Tpo/mic Ab & TgAb by indirect immunoflourescence techniques. The data was collected using a questionnaire and interviewing with subjects. Results: 82.6% and 86.9% of the hypo-and hyperthyroid subjects were positive for thyroid Abs, respectively. They had either positive Tpo/mic Ab or TgA b or both. Controls showed only 6% seropositivity. Thyroid auto Abs in females (89.2%) were significantly higher than those in males (10.8%). These Abs were most frequent in 3rd-4th decade of life .Thyroid Abs seroprevalence was significantly higher in Fars ethnic group than in Arab group. Conclusion: Our findings indicate that the occurrence of thyroid autoimmunity is common among the hypo- and hyperthyroid patients in Ahwaz city. Further studies to assess the causes are recommended.

p-877
FABRICATION OF GELATIN SPONGE REINFORCED WITH POLYPROPYLENE/ POLYETHYLENE TEREPHTHALATE ALLOY FIBERS AS TISSUE ENGINEERED SCAFFOLD

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This study focuses on mechanically reinforced gelatin scaffolds. In this experiment, Polypropylene/Polyethylene Terephthalate (PP/PET) alloy fibers were used for reinforcement. In order to obtain gelatin sponges incorporating PP/PET fiber to various extents, first, PP/PET fibers with weight ratios of 0.5, 1, 1.5, 2.5 and 5 were dispersed into gelatin solution, homogeneously and freeze-dried. Then, dehydrothermal cross-linking was applied to the resulting samples. Scanning electron microscopy observations of the resulting gelatin scaffolds indicated isotropic and interconnected pore structures with the average pore size of 212.04 micrometer that are necessary for cell culture. Therefore, PP/PET fiber incorporation enabled the gelatin scaffolds to enhance their compression strength significantly.

The process mentioned in this paper may provide vast applications suitable for cell culture and related fields.

p-878
A COMPARISON OF THE EFFECT OF MENTHA PULEGIUM LIQUID EXTRACT AND VITAMIN E ON BLOOD ANTIOXIDANT CAPACITY

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Many medicinal herbs have been presented for their potential use as alternative remedy for the treatment of many infections and the preservation of food from the toxic effects of oxidants. It is suggested that the preservative effect of Mentha pulegium is due to the presence of antioxidative and antimicrobial constituents.In this research the effect of Mentha pulegium liquid extract was compared with the effect of vitamin E on the blood oxidative stress. A clinical trial study on 60 subjects, randomly divided into 3 groups was designed. The first group consumed Mentha pulegium liquid extract with the dose of 3 g/day in 300 ml boiling water and the second group consumed vitamin E with the dose of 400 IU/day and the third one was the control group. Before and after two weeks of intervention, 5ml of blood were obtained from all groups and analysed for antioxidant capacity by FRAP method with the use of TPTZ indicator. In this method the descriptive analysis (mean± SD) and analytic analysis (ANOVA) were used. The rate of the serum total antioxidant capacity (mean ±SD) in the first, the second and the third group before and after was as follows: before: 1.9 ± 0.326, 2.2 ± 0.1, 2 ± 0.7 and after: 2.3 ± 0.5, 2.9 ± 0.2, 2.1± 0.8, respectively. The results showed a significant difference between the first and the second groups. The results indicated that Mentha pulegium has antioxidant activity; however this activity was lower than the one found in vitamin E.

p-879
SPLEEN HISTOPATHOLOGY OF SUB-CHRONIC DERMAL TOXICITY OF NANOSILVER IN GUINEA PIGS

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The colloidal nanosilver solution particles have a size of <100 nm in diameter. The colloidal nanosilver solution can be used in sanitary products, solutions for cleansing agents for clothings, and soaking solutions for toothbrushes. It can also be used in health care products, treating patients with all kinds of injuries and/or burns, bacterial and fungal infections, gastrointestinal l infections, and sexually transmitted diseases.In this study sub chronic dermal toxicity and variations of macroscopic effect on skin were investigated. In the assessment of sub- chronic dermal toxicity, we used three different doses of nanosilver and one concentration of a solution of 0.5% AgNO3. In this test, we used 2 control groups (positive, negative) and 3 test groups. The experiments were performed for 8- 13 weeks, and 5 days per week. In this study, histopathological changes of the spleen were investigated. These changes were maximal in the nanosilver concentration of 10000 ppm compared to other concentrations.

p-880
ASSOCIATION BETWEEN BLOOD DONATION AND ANTIOXIDANT ENZYMES AND LIPID PEROXIDATION IN 30-60- YEAR OLD MEN CONSULTING TEHRAN BLOOD TRANSFUSION CENTERS

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Iron is a pro-oxidant cofactor that may be linked to atherosclerosis progression. Free iron catalyzes the generation of free radicals and free radicals promote the oxidation of lipids. Reduction of body iron stores secondary to blood donation has been hypothesized to reduce lipid peroxidation .The aim of this study was to evaluate the association between blood donation and antioxidant enzymes and lipid peroxidation product malondialdehyde(MDA) .We investigated hemoglobin(Hb), serum ferritin ,MDA level , glutathione peroxidase(GPX) and superoxide dismutase(SOD) activities in the whole blood of 150 male volunteer blood donors aged from 30 to 60 years attending Tehran blood transfusion center. Subjects were divided into 5 groups according to the frequency of blood donation per year. With increasing the number of blood donations in a year the body iron stores , GPX activities and serum MDA of persons were significantly reduced ($p<0.05$) but SOD was significantly increased($p<0.05$). High-frequency blood donors had evidence of decreased body iron stores, decreased lipid peroxidation and enhanced activity of antioxidant enzymes when compared with low-frequency donors.

p-881
THE EFFECT OF CINNAMON ON BLOOD OXIDATIVE STRESS IN RADIOLOGY STAFF

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Excessive production of reactive oxygen species has been observed because of acute and chronic exposure to radiation which can lead to several detrimental and irreversible outcomes in the vital organs. Many plants contain natural antioxidants that function in metabolic response to the endogenous production of free radicals and other oxidant species. Cinnamomum zeylanicum is one of the important spices and aromatic crops that is used in the traditional medicine. The aim of this study was to determine the effect of cinnamon on oxidative stress status in radiology unit workers who are exposed to persistent low- dose radiation. A clinical trial was designed. A group of 27 radiology unit employees was demanded to consume C. Zeylanicum (0.5 gr/300 ml boiling water) twice daily for 14 days. Before and after intervention the lipid peroxidation level (LPO), total antioxidant capacity (TAC) and total thiol (SH) molecules were measured in the blood samples. In this method the descriptive analysis (mean± SD) and analytic analysis (paired t-test) were used. After intervention, a significant reduction of

blood LP0 (5.18± 6 versus 2.89 ± 2.09, p= 0.044) was observed. The total thiol molecules (0.125± 0.091 versus 0.17 ± 0.088, p= 0.067) and TAC (1.74 ± 0.37 versus 2.76 ± 0.87, p= 0.0001) increased but the increase of total thiols was not significant. Cinnamon has marked antioxidant potential and may be beneficial in alleviating many illnesses complications related to oxidative stress in radiology staff.

p-882
IRON DEFICIENCY IN PREGNANT WOMEN IN ESLAMSHAHR

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Introduction: Anemia in pregnancy is a significant public health problem especially in developing countries. Iron deficiency anemia in pregnancy may lead to increased risks of preterm delivery, low birth weight and still birth. This study was undertaken to detect iron deficiency anemia in pregnant women referring to health care centers in Eslamshahr /Iran. Methods: In this study, 266 selected pregnant women were divided into three groups of 1st trimester (n=58), 2nd trimester (n=73) and 3rd trimester (n=135) and their venous blood samples were collected for the determination of hemoglobin (Hb). Hematocrit (Hct), MCV, serum iron, TIBC, transferrin saturation (TS) and serum ferritin. Data were collected using a general information questionnaire and daily intake of energy, protein and iron were estimated using 24hr dietary recall questionnaire. These data were analyzed by frequency, mean and standard deviation and chi square tests, Anova and Tukey HSD. Results: Compared to normal levels, in the first trimester 1.7%, and 40.4%, in second trimester 5.7%, and 49.7%, and in the third trimester 2.8%, and 54.5% of pregnant women were deficient in Hb and serum iron, respectively.Also in the first trimester 54.5%, in the second trimester 10.4% and in the third trimester 22.7% of pregnant women were deficient in daily iron intake, respectively. Conclusion: The prevalence of iron deficiency anemia in the studied population is high. Hb determination is not enough to detect iron deficiency anemia and ferritin determination is necessary to assess iron stores. Moreover, iron supplements can not completely treat anemia.

p-883
PROTEIN PRECIPITATION METHODS EVALUATED FOR DETERMINATION OF SERUM NITRIC OXIDE END PRODUCTS BY THE GRIESS ASSAY

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Deproteinization is a necessary step in the measurement of serum NOx (nitrite+nitrate) concentrations. This study aims at comparing different protein precipitation methods for measurement of serum nitric oxide end products concentrations by the Griess method. For this purpose ten protein precipitation methods, most of which had been previously used for deproteinization in serum NOx determination, were used for protein removal. Serum samples from healthy volunteers were deproteinized with different methods and serum NOx concentrations were determined by the Griess assay. Pearson correlation coefficients and mean differences between values were determined for each method (using ultrafiltration as a reference method). P-values less than 0.05 were considered significant. Nitrite standard curve had linearity up to 150 µmol/l (r2=0.998). Our results showed that acid solutions are not suitable for protein removal in serum NOx determination. Using methanol, ethanol, and diethylether/methanol resulted in higher serum NOx values. Acetonitrile and zinc sulfate had good agreement with ultrafiltration method for serum NOx determination in terms of their mean differences from ultrafiltration method (-1.6 ± 8.6 and -2.3 ± 7.4 respectively). It can be concluded that zinc sulfate and acetonitrile are the methods of choice for protein removal in determining serum NOx concentrations by the Griess method.

p-884
PREPARATION OF RECOMBINANT ANTI-DIGOXIN ANTIBODY USING PHAGE DISPLAY TECHNOLOGY AND ITS COMPARISON WITH MONOCLONAL HYBRIDOMA ANTIBODY

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The cardiac glycoside digoxin is widely used for the treatment of congestive heart failure and cardiac arrythmias. Digoxin is a highly toxic drug and consequently is routinely measured in sera of treated patients. To produce recombinant single chain antibody against digoxin, the RNA from two sources of hybridoma cells and spleen cells of Balb/c mice immunized via injection of digoxin-BSA were used. The variable regions of the VH and VL of the repertoire of antibody genes were amplified by PCR. scFv constructs were generated by SOE-PCR. After purification, the scFv was digested by sfi1 and Not1, and was ligated into the phage display vector pCANTAB5E. The ligated DNAs were used to transform E.coli TG1 cells, in which the amber stop codon between the E tag DNA sequence and g3 was not read, allowing the production of scFv Etag-p3 fusion protein. Recombinant phage, expressing a library of scFv polypeptides on their surface, were produced by helper phage rescue and selectively enriched by 5 rounds of panning. Phages with higher affinity for digoxin were selected following lowering antigen concentration using digoxin- BSA coated wells. After 5 rounds of panning, 100 individual clones were analyzed by ELISA.To produce soluble scFv, E.coli HB2151 cells were infected with selected phages. Finally the product was collected from the supernatant. Primary experiments showed that immunized animals give rise to better immunoglobins having higher affinity and binding properties. Hence the gene from the immunized mice was selected for further studies. The standard curve was constructed following a competitive procedure in a range of 100-1000,000 pg/ml. Sensitivity of mAb AR85 was determined to be about 270pg/ml while that for mAb was reported to be 100pg/ml.However the saturation value for recombinant mAb was found to be 100ng while that for mAb was reported to be 10ng. The affinity constant of

recombinant mAb AR85 towards digoxin was also found to be ka=3.8×10 ^{7} M^{-1} while that for mAb was reported to be ka=2.6×10^{8} M^{-1}.

p-885
REFOLDING STUDIES OF HUMAN INOSINE TRIPHOSPHATASE (hITPASE) BY AN "ON COLUMN CHROMATIC" METHOD

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Genomic DNA is regularly damaged by endogenous or exogenous damaging agents. In contrast, all of the cells are equipped with some defense systems to protect the integrity of their genetic information. Oxidative deamination is one of the most important types of damage which cause genetic mutations such as base substitutions. (Deoxy) inosine triphosphate ((d) ITP) and xanthine triphosphate (XTP) are the main products of oxidative deamination of free purine nucleotides which can be incorporated into DNA and RNA by polymerases and cause mutations and disrupt the expression of genetic information. It has been proposed that hITPase which is encoded by ITPA gene, located on human chromosome 20 is able to remove deamination of rough purine nucleotides from nucleotide pool of cells by hydrolyzing them to their corresponding mono-phosphate forms. Therefore, hITPase may be considered as one of the defensive enzymes that protect genetic information. In this study, hITPase was expressed in E.coli BL21. Purification of recombinant protein was done using affinity chromatography column. Refolding of hITPase was studied using an "on chromatography column" method. The precise refolding of the protein was investigated by PAGE and circular dichroism techniques. In this report, we represent our findings about structure and refolding of recombinant hITPase.

p-886
ASSOCIATION BETWEEN BLOOD DONATION AND IRON STATUS IN 30-60 YEARS OLD MEN CONSULTING TEHRAN BLOOD TRANSFUSION CENTERS

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Bleeding results in mobilization of iron from body stores. With continued bleeding an individual either reaches equilibrium at a lower level of iron stores or becomes anemic. The aim of this study was to evaluate the effects of blood donation, and frequency over a year's time, on iron status. We investigated hemoglobin (Hb), serum ferritin , serum iron (SI), hematocrit (Hct) ,total iron binding capacity (TIBC), and transferrin saturation(TS) in the whole blood of 150 male volunteer blood donors aged from 30 to 60 years attending Tehran blood transfusion center. Subjects were divided into 5 groups according to the frequency of blood donation per year. With increasing the number of blood donations in a year the levels of Hb, Hct, and iron status indices were significantly reduced but TIBC was significantly increased. Frequency of blood donation per year was also inversely correlated with Hb (r = -5.54 , P <0.001) , serum ferritin (r = - 6.01 , P <0.001) ,Hct (r=-3.81 , P<0.001), SI(r=-4.21, P<0.001) , and TS (r= -4.49 , P<0.001), but was directly correlated with TIBC (r=3.29 , P<0.001) . Serum ferritin showed significant correlation with age (r=0.181, P<0.05) .From the data obtained it would appear that male donors, while depleting their iron stores, were able to donate 2-3 U/year without an appreciable incidence of iron deficiency.

p-887
ISOLATION AND PRIMARY CULTURE OF UROTHELIAL CELLS FROM CANINE BLADDER

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Urinary bladder is susceptible to a variety of pathological conditions from the time of fetal development to adulthood. Aside from congenital abnormalities, other disorders may lead to bladder damage or loss, requiring eventual reconstruction. The tissues used for reconstruction may lead to complications due to their inherently different functional parameters. In most cases, the replacement of lost or deficient tissues with functionally equivalent tissues would improve the outcome of the patients. Tissue engineering techniques are very useful for attaining this goal. For many years researchers have tried to reconstruct the damaged tissues with the normal ones engineered by different ways to retain their original functions. We have set up the isolation and culture of canine bladder urothelium successfully. Briefly transmural sections of canine bladder tissue were aseptically obtained. The samples were placed into calcium and magnesium free HBSS containing 10 mM HEPES and incubated at 4°C overnight to release the cells. The cultures maintained in keratinocyte serum free medium (KSFM), which discouraged growth of non-epithelial cells and only urothelial cells became established in the culture. The medium was replaced after 24 h and subsequently on alternate days. Primary urothelial cells were propagated in tissue culture plates. These cells grew as non-stratified monolayers and immunocytochemistry for cytokeratin 8 and 18 confirmed the urothelial nature of these cells.

p-888
EFFECTS OF OF THE PRETREATMENT WITH SECURIGERA SECURIDACA (L.) SUSPENSION ON GLUCOSE, INSULIN AND TOTAL SERUM ANTIOXIDANT LEVELS AFTER ALLOXAN ADMINISRATION TO RATS

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Interest has grown in the usage of natural antioxidantsas a new strategy for alleviating the oxidative damage in primary stages of diabetes. Recently, Securigera securidaca (L.) seeds are used as an antidiabetic agent in India & Iran. Phytochemical analysis showed that the S. securidaca (L.) seed extracts were rich in flavonoids and so they have antioxidant properties. The present study was thus undertaken to assess the protective effect of S. securidaca (L.) seed suspension on oxidative damage induced by alloxan in rat pancreatic tissue and their possible role in ameliorating the development of type I diabetes mellitus. S. securidaca (L.) seeds were milled and dissolved in distilled water. Totally 90 rats were used and divided into three groups: 1) control rats 2) rats using a high dose of S. securidaca suspension (4g/kg/d) (3) and rats using a low of S. securidata (2 g/kg/d). Rats in 2 & 3 groups were pretreated with S. securidaca suspension by gavage prior to subcutaneous injection of alloxan. After 24h, 48h, and 72h of alloxan injection ,serum glucose (by commercially kit), serum insulin levels (by ELISA kit) and serum total antioxidant power (by FRAP assay) were measured. This study showed that oral adminstration of S. securidaca (L.) (both low and high doses) significantly inhibited the increase of serum glucose caused by alloxan ($p<0.05$) Also high and low doses of S. securidaca (L.) caused a significant increase in serum insulin levels in pretreated rats in 24h, 48h, and 72h ($P<0.05$). Measurement of serum total antioxidant levels revealed that this value increased in preteated rats proportional to control groups ($p<0.05$). It seems that S. securidaca (L.) seed suspension decreases β-cell damage due to their high antioxidant capacity and their effects on antioxidant system. In addition, the use of this suspension can directly induce insalin release from the remaining pancreatic β-cells.

p-889
ECDYSTEROID RELEASE BY THE PROTHORACIC GLAND AND PTTH mRNA LEVELS OF THE CRICKETS

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Ecdysteroids and peptide hormones control a variety of developmental and behavioral processes in insects. It was generally accepted that prothoracic glands produce only ecdysone. Recently it has been shown that molting glands of some insects also synthesize 20-hydroxyecdysone other than alpha ecdysone. In the present study we wanted to find out whether the glands of the cricket, Gryllus bimaculatus, also produce 3-dehydroecdysone. The in vitro secretion of ecdysteroids from prothoracic glands was analyzed by high performance liquid chromatography (HPLC) and radioimmunoassay (RIA). The primary product was identified as 3-dehydroecdysone (70 to 85%) with fewer amounts of ecdysone. The nature of 3-dehydroecdysone was confirmed by its enzymatic conversion to ecdysone with haemolymph 3-dehydroecdysone 3β-reductase preparation. Furthermore, hemolymph ecdysteroid titers and mRNA levels were determined during the molt cycle. RIA of hemolymph samples revealed that the ecdysteroid titer is low during intermolt. PTTH mRNA levels were analyzed by Northern blotting of total RNA from corpus cardiacum-allatum complex. However, the peptide transcript was present in both organs. Nevertheless, quantification of peptide levels will be a necessary step in further understanding the role of hormone in the regulation of molting.

p-890
MGF-82 NANOPARTICLE, A NEW ADDUCT OF FULLERENE FOR DRUG DELIVERY

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Nanomedicine is beginning to emerge from research in nanotechnology. Nanotechnology refers to the scale of nanometers. Nanoscale materials often have novel properties related to their high ratio of surface area and quantum effects. Biopharmaceutics is one of nanomedicine applications. Efforts are focused on drug delivery applications using nanomaterial coatings to encapsulate drugs and also to serve as functional carriers. Nanomaterial encapsulation could improve the diffusion, degradation, and targeting of a drug. Furthermore, nanomaterials could serve as camouflage to avoid immune responses, or as agents which could catalyze or respond to certain molecules or chemical events. MGF-82 Nanoparticle, a new adduct of C60-fullerene, with the ability to serve as a $25Mg^{2+}$-releasing smart nanoparticle, is recently found to be a mitochondrial membrane tropic agent for mammalian myocardiocyte. Therefore MGF-82 Nanoparticle is a promising tool for activation of Mg^{2+}-dependent ATP production in vivo which is especially essential in case of numerous hypoxia syndromes, as it releases $25Mg^{2+}$ in response to acidosis. Taking the above information into account, the elucidation of the mitochondrial membrane located MGF-82 nanoparticle-specific receptor, is an important goal for scientific investigation to improve the medicinal efficiency of the drug studied. The results show the specific properties of the receptor that help to improve MGF-82 Nanoparticle targeting and selectivity which is a key problem of the contemporary molecular pharmacology.

p-891
L-ARGININE SUPPLEMENTATION INFLUENCES NITRITE BUT NOT NITRATE AND TOTAL NITRITE IN RABBIT MODEL OF HYPERCHOLESTEROLEMIA

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Background: The assessment of an altered nitric oxide (NO) availability is of potentially diagnostic and prognostic significance. The present study is aimed to investigate the

effect of L-arginine supplementation as an NO donor on NO metabolites in a rabbit model of hypercholesterolemia. This may lead to the discovery of a reliable marker for endothelial NO production. Methods : White male rabbits were randomly assigned in 2 groups. Rabbits were fed 1% high-cholesterol diet (HC group, n=10), or high-cholesterol diet with oral L-arginine (3% in drinking water) (HC+L-arg group, n=10) for 4 weeks. The serum levels of lipids, L- arginine, total NO metabolites (NOx), nitrite and nitrate were measured at the beginning and at the end of the study. Results: L-arginine supplementation led to significant increase in plasma level of L- arginine. The serum level of nitrite was significantly higher in L- arginine treated group, while serum level of nitrate and NOx were significantly lower than HC group. Conclusion: The results of our study showed that nitrite is a useful marker of endogenous endothelial NO production. Despite frequent use, neither nitrate nor NOx are reliable markers of acute changes in eNOS activity.

p-892

A STUDY ON THE INTERACTION BETWEEN MORIN AND RNA BY FTIR & UV SPECTROSCOPIC METHODS

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Morin (3, 2, 4, 5, 7- pentahydroxyflavone) is one of the most common flavonols and occurs in tea, coffee, cereal grains and a variety of fruits and vegetables. As a widely distributed biological active compound, morin has been reported to present many specific therapeutic activities and it has aroused considerable interest due to its broad pharmacological activity such as antitumor, antioxidant, antiviral, anticancer and antiallergic effects that prevent RNA damage, which is implicated as its mechanism of action. However there has been no information on the interaction of this antioxidant with RNA at the molecular level. The aim of this study was to examine the stability and structural features of RNA complexes with morin in aqueous solution at physiological pH, using a constant RNA concentration (0.1 mM) and various morin/RNA(P) ratios of 1/80, 1/40, 1/20, 1/10, 1/5, 1/2 and 1/1. FTIR, UV-Visible spectroscopic methods were used to determine the ligand external modes, the binding constant and the stability of morin– RNA complex in aqueous solution. Spectroscopic evidence showed that complexing of morin with RNA occurrs via G-C and A-T and PO2 groups with overall binding constants of K (morin-RNA) = 9.15×10^{3} M^{1-}.

p-893

SAFFRON CAROTENOID EXTRACTION USING A NEW CHELATING RESIN

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Crocus Sativus L. (Saffron) is used in Iranian and Chinese traditional medicine to treat some disorders of central nervous system (CNS). Crocin is the major biologically active ingredient of saffron reported to prevent ethanol-induced impairment of learning and memory, and to inhibit neuronal cell death induced by both internal and external apoptotic stimuli. Since crocin is relatively unstable and t is not available commercially, every investigator has to extract fresh crocin for use. We report, here, a simple, fast and reproducible column chromatographic method to prepare crocin. Methanol extract of dried stigma of saffron was vacuum evaporated. The product was loaded on a column of Amberlite XAD-7 resin. The columns were either washed with diethyl ether-methanol-dioxane, methanol-dioxane or with dioxane alone. UV/Vis. specterophotometric experiments showed that methanol-dioxane elution was more favorable compared to the other combinations, producing a higher yield of crocin in a shorter period of time.

p-894

INVESTIGATION OF THE ROLE OF ANGIOTENSINII (ANGII) IN REACTIVE OXYGEN SPECIES PRODUCTION AND THE MODULATORY ROLE OF NO IN VESSEL RESPONSES TO ANII IN ACUTE JOINT INFLAMMATION IN THE RABBIT

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Objective: It has been established that NO production in most tissues increases during acute inflammation. Regulation of joint blood flow (JBF) is important in this situation so the aim of this study was to investigate the interaction of local AngII and reactive oxygen species (ROS) production and the modulatory role of NO in regulation of joint blood flow during acute inflammation. Methods: The present study was performed on 24 New Zzealand white rabbits divided into three experimental and one control groups. Acute knee joint inflammation was produced by intraarticular injection of 0.5 ml of a 2% solution of carrageenan in knee joint. In the first group after 24 hours animals were anesthetized by thiopental sodium and carotid artery, jugular vein and saphenous artery were cannulated for recording blood pressure, injection of L-NAME and local injection of AngII and losartan, respectively. Blood flow was recorded by laser Doppler flow meter. Joint vascular resistance (JVR) was calculated by dividing arterial blood pressure (ABP) by (JBF). In the second group, knee joint tissue was used for homogenization and ROS measurement .In the third group, Losartan (10mg/kg) was administrated orally 2 hours before carrageenan. Result: JVR in response to AngII was significantly increased before and after L-NAME ($P<0.01$). Losartan completely blocked the effect of AngII on JVR.Data showed that total amount of antioxidant and catalase activity nonsignificantly increased in inflamed group. Losartan returned significantly the catalase activity to the normal level ($P<0.01$). Conclusion: It seems that NO plays a role in the regulation of joint vascular tone and modulates the AT1 receptor which mediates vasoconstrictor effects of AngII on these vessels. The inhibition of increase in ROS production by losartan indicates that the production of ROS during joint inflammation is via angiotensinII and through the AT1 receptors. Key words: Nitric oxide, Reactive oxygen species, acute inflammation, knee joint.

p-895
ALTERATIONS OF ANTIOXIDANT ENZYMES INDUCED BY SALINITY IN TISSUE CULTURES OF TRIGONELLA SPECIES

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Plants in natural conditions of growth and development are inevitably exposed to different types of stresses, which may cause increased production of active oxygen species. These include superoxide radicals ($O2^-$), singlet oxygen ($^1O_2$), hydrogen peroxide (H2O2) and hydroxyl radicals (OH^o),which cause tissue injury. In plant cells the protective mechanisms to eliminate these is by an antioxidant system like SOD, POX, and PPO enzymes. Trigonella belongs to the large family of Fabaceae and has important medicinal properties. Salinity is a major abiotic stress affecting the metabolism and yield of crops worldwide. In this study we determined the activity of some antioxidant enzymes and the protein content in the calli of four endemic Trigonella species (T.elliptica,T.tehranica,T.aphanoneura and T.foenum-graecum) under different concentrations of NaCl (0, 50, 100, 150, and 200 mM). The enzyme activities were determined by spectrophotometric methods and isoforms of some antioxidant enzymes were characterized by PAGE and SDS-PAGE electrophoresis system. In some species the activity of POX increased significantly with an increase of salinity. The protein content mostly increased.

p-896
COMPARISION OF BYSMV CONSERVED BLOCKS OF L GENE WITH OTHER PLANT RHABDOVIRUSES

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Barley yellow striated mosaic virus (BYSMV) is the member of the genus Cytorhabdovirus in the family Rhabdoviridae. It causes mosaic and chlorotic striation on wheat, barley, millet and other gramineous plants and is transmitted by the plant hopper Loadelphax striatellus in a propagative manner. The virus has been reported from European countries and Iran. Despite its widespread occurrence, no information is available regarding its molecular properties and phylogenetic relationship. In the present work, information is presented on the nucleotide sequence and deduced amino acid content of the polymerase (L) gene. Among the five or more open reading frames (ORFs) in rhabdoviruses, the L is the largest. For isolation of the viral nucleocapsid, freshly infected barley tissues were homogenized in 0.1 M sodium citrate buffer, pH 6.5, followed by differential centrifugation. The nucleotide sequences of L gene of Iranian BYSMV isolate was determined using a random-PCR method (rPCR) followed by PCR with specific primers. Deduced amino acid sequences of the L gene were aligned with those of other members of Rhabdoviridae obtained from the GenBank using CLUSTAL W software and a cluster dendrogram was depicted. It revealed conservation of sequence within 6 blocks that appear sequentially along the protein. A cluster dendrogram derived from the L protein alignments indicated that BYSMV is more closely related to northern cereal mosaic cytorhabdovirus than to other plant rhabdoviruses.

p-897
COMPARATIVE EFFECTS OF L-CARNITINE AND ACETY-L-CARNITIN E WITH PENTOXIPHYLIN IN MOTILITY AND QUALITY OF MOUSE TESTICULAR SPERM

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Background: One of the effective elements that has impacts on assisted reproductive thechnology is to obtain sperm with good quality. Testicular sperm extractin is used to treat Azospermia .Howevere, these sperms are immature and immotile. Therefore, it is important to find some chemicals to improve sperm quality and to provide natural conditions that one finds in epididymidis. Therefore, the objectives of this study were to find the effects of L-carnitine and acety-L-carnitine on quality and motility of testicular sperm and to compare it with those of pantoxyphilin. Materials and methods: Sperms were extracted from 30 male mice. The sperm samples were divided into four aliquots with one control and three experimental groups. The experimental groups were exposed to 1.76 mm of L-carnitine, acetyl-L-carnitine and pentoxyphilin, respectively. Every 30, 90 and 180 min. sampling were done and smears were prepared. The smears were stained with aniline blue, chromomycin A3 and acridine orange. The sperm motility was also evaluated. Results: The results indicated that sperm motility increases in all experimental groups. However, L-carnitine, and acetyl-L-carnitine also improved the sperm chromatin qualities, pentoxyphilin just improved sperm motility but not sperm chromatin quality. Conclusion: Although it was shown previously that L-carnitine improves sperm motility and maturation in vivo, this study indicated that L-carnitine and acetyl-L-carnitin could induce sperm chromatin maturation in vitro. L-carnitine and acetyl-L-carnitine produced better results than pentoxyphilin, because pentoxyphilin only improves sperm motility, while L-carnitine, and acetyl-L-carnitine increase the percentage of both motile and mature sperms.

p-898
SERUM PARAOXONASE-1 ACTIVITY AND GENE POLYMORPHISMS DETECTED IN HYPERLIPIDEMIC DIABETIC PATIENTS

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Paraoxonase-1(POX1), an anti-atherosclerotic factor, has various polymorphisms. The aim of our study was measurement of enzyme activity and determination of PON1 polymorphisms in hyperlipidemic diabetic patients. Twenty five hyperlipidemic diabetic patients (11 females and 14 males) and seventy five healthy subjects (36 females and 39 males) were included in our study. PON1 activity was measured using paraoxone and phenylacetate as substrates. The enzyme polymorphisms were determined by specific-allele PCR. Serum paraoxonase activity was significantly reduced in diabetic patients ($p<0.05$). PON1 genotype distribution and allele frequencies were determined in diabetic group (QQ=60%, QR=40%, RR=2%) and healthy subjects (QQ=46%, QR=39%, RR=15%). Decreased activity of PON1 in diabetic group may be due to low HDL concentration and its biochemical modification (glycation).

p-899
PROOXIDANT-ANTIOXIDANT BALANCE AS A NEW RISK FACTOR IN PATIENTS WITH ANGIOGRAGHICALLY DEFINED CORONARY ARTERY DISEASE

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Several evidences support a role for oxidative stress in atherogenesis. Oxidative stress is defined as an imbalance between prooxidants and antioxidants in favor of prooxidants. In this study the prooxidant-antioxidant balance (PAB) in patients with angiograghically defined coronary artery disease (CAD+) was determined by a modified PAB assay and presentation of PAB value as a novel cardiovascular risk factor. For sixty-one patients with CAD+ and sixty-three healthy volunteers, the PAB were measured and its correlation was determined with anthropological and clinical parameters. A significant increase of the PAB value was observed in patients in comparison to control group. A correlation, which is not quite significant, was noted between angiographic findings (number of diseased vessels) and the PAB values in patients. A significant positive correlation was established between the PAB value and systolic blood pressure, diastolic blood pressure, smoking, fasting blood sugar and serum urea concentration; and a significant negative correlation was established between PAB value and serum creatinine. These results indicate that the PAB value may be considered as a cardiovascular risk factor. Further clinical research is needed to substantiate the potency of the PAB value as a cardiovascular risk factor.

p-900
THE ANTIOXIDANT ACTIVITY OF ZHUMERIA MAJDAE

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Zhumeria majdae "Mohr-e-khosh" is one the endemic species from Labiate family which has a limited geographic range in southern region of Iran (near Persian Gulf). In Hormozgan Province (south of Iran), people use Zhumeria majdae as a hypoglycemic, hypolipidemic, antiseptic and analgesic agent. Zhumeria extract showed free radical (DPPH) scavenging capacity (IC50 of Zhumeria crude extract was more than 400 µg/ ml while IC50 of BHT was 63.17 µg/ ml. Zhumeria crude extract could not inhibit the peroxidation of β-carotene but its ethyl acetate and butanolic fractions inhibited the peroxidation of β-carotene with antioxidant activity coefficients (ACC) of 295.2 ± 28.2 and 189.2 ± 27.37, respectively. The ACC of BHT and gallic acid were 866.6 and 754.03 µg/ ml. The content of total phenolic compounds in Zhumeria crude extract was determined by Folin-Ciocalteu method. The results of this research show that this extract has free radical scavenging and lipid peroxidation attenuation capacity. Zhumeria majdae seems to be useful in treatment of human pathological conditions in which free radical production plays a key role.

p-901
EFFECTS OF HYPERTENSION ON DNA METHYLATION STATE OF THE UTERUS

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Hypertension is a risk factor for many of the uterine disorders like hysterectomy. Several genes that involve in blood pressure regulation have methylated promoters. Methylation of DNA is an epigenetic process which modulates gene expression. To examine whether the uterine methylation status of the DNA content is modifed in hypertensive rats, we compared stroke-prone spontaneously hypertensive rats (SHR) and Sprague-Dawley rat uterine epithelium and glands. For this purpose, uterus of the SHR and SD rats were sectioned and stained with 5 methylcytosine antibody. The staining intensity was evaluated by scion image analyses software. The data were analyzed with Mann-Whitney U test. We also measured the glutathione content of the uterus by HPLC. The

results indicated that the amount of 5 methyl cytosine content of the DNA of epithelium and glandular cells of SD rats are more than that of SHR rats. However, there was no significant difference in uterine glutathione content of two rat strains. It seems that hypomethylation of the DNA can modify the gene expression pattern of uterine cells and this may lead to some kinds of disorders in uterus. This DNA modification in uterus may be related to hypertension.

p-902
EFFECT OF HYDROALCOHOLIC EXTRCT OF ANETHEUM GRAVEOLENS ON STRUCTURE OF KIDNEY IN FEMALE RAT

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Anethum Graveolnes is one of the medicinal plants which is used for digestion problems, it has been reported that the long term use of this plant in large doses, might cause decrease urine. It seems this plant has side effect in urinary system. Therefore the aim of this study was to evaluate the effect of hydroalcoholic extract of Anethum Graneolens on kidney in Rats. For this purpose, forty rats were divided into 3 experimental and control groups. Experimental groups were given orally 10, 50,100 mg/kg of extract for 45 days. The same volume of saline was given to control group. After termination of the experiments, the kidney of all mice were removed under deep anesthesia and stained with H&E after histological processing of the tissue. The results show that,the extract of Anethum Graveolnes had no histological effect on the microscopic structure of the kidney, but morphometrical analysis revealed that glomerular diameter and epithelium height were significantly decreased. In conclusion, it seems that Anethum Graveolnes might be able to cause some side effect on kidney in long term and this leads to renal dysfunction. but further studies are required to clarify the issue.

p-903
EMBRYONIC STEM CELL SPHERE: A CONTROLLED METHOD FOR PRODUCTION OF EMBRYONIC STEM CELL AGGREGATES FOR DIFFERENTIATION

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Embryonic stem cells are of significant interest as a renewable source of non proliferating cells. The basic strategy for in vitro differentiation of embryonic stem cells is the formation of multicellular aggregates called embryoid bodies (EBs). Standard methods for EB formation (hanging drop and spontaneous aggregation of ESC in suspension culture) are limited in their production capacity and varying aggregate size. They also are not easily amenable to process-control strategies (the need of frequent passages). The encapsulation of ES cells in hydrogel would overcome many of these current culture limitations. In this study, we immobilized ESCs into alginate beads as the scaffold bioreactor system to generate scalable quantities of undifferentiated ES aggregates with narrow distribution in size and to facilitate process control strategies for differentiation compared to 2D culture conditions for EBs production (hanging drop and spontaneous aggregation of ESCs in suspension culture). mESCs line Royan B1 were resuspended in alginate solution, then the alginate/cell suspension were addedrop wise into CaCl2 solution to form beads. Alginate beads containing ESCs were transferred to 12-well plates. From day 3 postencapsulation, embryonic spherical structures called ESC-spheres were observed in each well. To assess growth profile of ESCs in alginate, the numbers of ESC-spheres were counted for 20 days. Expression of Oct4 and SSEA1 in ESC-spheres at 3, 7, 10 and 14 days postencapsulation was considered as undifferentiated indicators of ES cells and analyzed by flowcytometry. The results were compared to ESCs (Royan B1) as control group. The utility of ESC-spheres for differentiation was determined by generation of cardiomyocytes and neurons. The released ESC-spheres from alginate beads were differentiated into cardiomyocytes and neurons while EBs from hanging drop and spontaneous aggregation of ESCs in suspension were used for differentiation as control groups. Cardiac and neural markers were assessed by immunostaining to confirm cardiomyocytes and neurons. Expression of cardiac and neural specific genes was determined by RT-PCR. The results of immunostainig and RT-PCR were compared to cardiomyocytes and neurons derived from control groups (EBs from hanging drop and spontaneous aggregation of ESCs in suspension). First a basic understanding of ESCs behavior in alginate beads was acquired by counting of ESC-spheres. The results revealed that from day 3 postencapsulation (initial lag phase), the numbers of ESC-spheres progressively increased for about 10 days. Flowcytometric analysis of ESC-spheres at days 3, 7, 10 and 14 postencapsulation in alginate, showed high expression of Oct4 and SSEA1 in ESC-spheres as well as ESCs (Royan B1). The results of immunostaining showed the expression of cardiac (α-actinin, Connexine 43) and neural (MAP2, β-Tubulin III) markers in cardiomyocytes and neurons from ESC-spheres as well as control groups. The results of RT-PCR showed high expression of cardiac β-MHC, ANF, and MLC2V and neural PAX6, NKX2.2, NKX6.1, β-Tubulin III, and Mash1 specific genes in cardiomyocytes and neurons from ESC-spheres and EBs respectively. In summary we have demonstrated that alginate encapsulation provides a scalable system for production of undifferentiated ESC-spheres for differentiation. Alginate beads might be as a simple bioreactor which can produce a scalable system for production of undifferentiated ESC-spheres with narrow distribution in size without passaging conditions for differentiation. It may facilitate experimental designs for EB formation in a stem cell laboratory.

p-904
THE PROTECTIVE ROLEPLAYED BY ANTIOXIDANTS IN CYCLOSPORINE A-INDUCED OXIDATIVE STRESS IN RAT LIVER: THE MORPHOLOGICAL STUDY

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The immunosuppressive agent cyclosporine A (CsA) has been reported to exert measurable hepatotoxic effects. One of the causes leading to hepatotoxicity is thought to be reactive oxygen radical formation. In this study the potential of quercetin (Q) and vitamin E (vit.E), as antioxidants, in attenuating CsA-induced liver dysfunction in rats were investigated. Rats were divided into six groups, which were treated with either olive oil, ethanol + olive oil, CsA, CsA + vit.E, CsA + Q, or CsA + vit.E + Q for both 4 and 8 weeks. At the end of the treatments, the animals were sacrificed and hepatic tissue was treated for morphological (haematoxylin-eosin) and biochemical [reduced serum total protein, catalase (CAT) and glutathione peroxidase (GPx) and increased alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP)] analyses. The results indicate that CsA-induced hepatotoxicity was characterised by morphological alterations in tissue architecture, changes in GPx, MDA level and CAT activity. In conclusion, our data suggest that the imbalance between production of free oxygen radicals and antioxidant defence systems, due to CsA administration, is a mechanism responsible for oxidative stress. Moreover, we show that combination of the two antioxidants, Q and vit.E, play a protective action against CsA-induced oxidative stress, as supported by biochemical and morphological results.

p-905
EFFECTS OF CARROT SEED EXTRACT ON THE LIPID PEROXIDATION, TOTAL ANTIOXIDANT STATUS AND PAROXANASE (PON1) LEVELS OF MALE RATS

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The pathology of numerous chronic diseases, including cancer and heart disease, involves oxidative damage to cellular components. Antioxidants have been detected in a number of food and agricultural products, including cereal grains, vegetables, fruits and oil seeds. This study was conducted to investigate the effects of carrot seed extract (CSE) on plasma lipid peroxidation (LPO), total antioxidant status (TAS) and paroxonase (PON1) level of male rats. In this investigation, 40 male rats were divided into five groups, and 0 (control), 200, 400 and 400 (+5 mg/kg gentamycin) mg/kg /day of CSE was injected intra-peritoneally into groups 1, 2, 3 and 4 for 4 weeks, respectively. Group 5 received only 5mg/kg/day of gentamycin intraperitoneally. The plasma level of TAS in group two was more than the control group, and in group three it was more than groups two and control ($P<0.05$). Gentamycin had caused a remarkable decrease in the plasma level of TAS in group 5, however using CSE prevented such dramatic decrease ($P<0.05$). In spite of increasing TAS, the level of MDA only showed a significant difference between group 4 and 5 ($P<0.05$) and in the other groups the results were the same ($P>0.05$). Statical analysis revealed that the CSE had a significant effect on the level of PON1 plasma levels, e.g. there was a significant difference between the plasma level of PON1 in groups 3 when compared with the other groups ($P<0.05$). In conclusion, using CEA supplement in group 4 suppressed the decrease of this mentioned enzyme.

p-906
EVALUATION OF CARROT SEED EXTRACT ON THE SPERMATOGENESIS OF WISTAR RATS

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In ancient medicine the beneficial effect of carrot and its seeds on the impotency of the males has been mentioned .Hakim Sayed Esmail Jorjani has been written in carrot chapter of Zakhire Kharazmshah that carrot decreases impotency and its seeds are more powerful, and wild carrot is still more effective in men, but in females, it has antifertility effects. The study designed to determine the effect of carrot seed extract (CSE) on spermatogenesis and plasma levels of LH, FSH and testosterone. 40 Wistar male rats were divided into five groups, 0 (control), 200, 400 and 400 (+5mg/kg gentamycin) mg/kg/day CSE were injected intraperitoneally in groups 1,2,3 and 4 for 4 weeks, respectively. Group 5 received only 5 mg/kg/day gentamycin intraperitoneally. After 24 days, blood samples were collected and testes were excised and weighed and the number of the sperm cells in the homogenate of testis was counted. The results were evaluated nonparametically. The results showed that the injection of CSE causes significant increase in the concentration of sperm cells ($P<0.00$) while it had no effect on the vitality and motility of the sperms ($P>0.05$).Increasing the dosage of the CSE had no meaningful effect on the mentioned parameters. However 400 mg/kg/day of the CSE protected spermatogenesis from the inhibitory effect of gentamycin. Significant increase in the plasma levels of LH and testosterone were observed in the third group. In addition CSE prevents the negative effect of antibiotic on the production of the sex hormones and no marked differences in the serum levels of testosterone and LH were noticed between first and fifth groups.

p-907
ALL-TRANS RETINOIC ACID, 13-CIS RETINOIC ACID AND RETINOL IN SERA OF CHILDREN FROM IRAN

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Retinol is converted to biologically active and inactive metabolites, such as all trans retinoic acid (RA) and 13-cis retinoic acid respectively, within tissues. This study was designed to investigate the relationship between serum concentrations of retinol and its metabolites, RA and 13-cis retinoic acid. An HPLC method was used to assess retinol levels in the sera of 280 children who comprised the study population. Another HPLC method was employed to measure the levels of RA and 13-cis retinoic acid in the sera of four children who had retinol levels of <0.70 μmol/L and tested positive for relative dose response test. RA and 13-cis retinoic acid were also measured in sera of 67 healthy children randomly selected from the population under study. The mean RA level in sera of four children with marginal vitamin A deficiency (5.62± 4.00 nmol/L) was 2.5 fold over that of the healthy subjects in study population (2.18 ± 1.73 nmol/L). The difference between the two means was statistically significant ($p<0.05$, Mann-Whitney test). Serum levels of RA correlated negatively with those of retinol ($r = -0.745$, $p<0.001$). An exponential regression equation provided the optimum fit for the curvilinear inverse relation between serum retinol and RA. There was no relation between the levels of 13-cis retinoic acid and retinol. Serum RA levels might thus be elevated in vitamin A deficiency to compensate for the inadequacy of retinol in the circulation.

O-908

EFFECTS OF ADRENOMEDULLIN AND CALCITONIN GENE RELATED PEPTIDE ON C-FOS EXPRESSION, RELEASE OF NEUROTRANSMITTERS AND CAMP ACCUMULATION IN THE RAT SPINAL CORD

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Adrenomedullin (AM) immunoreactivity and mRNA, in addition to a large number of specific AM-binding sites, exist in the rat spinal cord. However, no phenotype has been reported for AM in the spinal cord. The objectives of this study were to investigate the effects AM and calcitonin gene related peptides (CGRP) on the expression of c-fos in the spinal cord of conscious rats, release of glutamate, aspartate, glycine, GABA and serotonin from slices prepared from the rat spinal cord and on the accumulation of cAMP in the embryonic spinal cells. AM induces c-fos expression in the rat spinal cord when administered intrathecally, with the pattern being similar to those produced by i.t. CGRP. Effects of the two peptides were sensitive to CGRP8-37(CGRP1 receptor antagonist). AM (10^{-7} and 10^{-6}M) had no significant effects on the basal and K+-evoked release of serotonin and the amino acids tested in this study. CGRP (10^{-7} and 10^{-6}M) also did not show any significant effects on the basal and K+-evoked release of serotonin, GABA and glycine. However, CGRP increased the K+-evoked release of aspartate and glutamate. Cellular levels of cAMP were increased by AM and less potently by CGRP. CGRP-induced cAMP accumulation was effectively inhibited by CGRP (8-37) (pA2 7.63 +/- 0.44) and hAM (22-52) (pA2 6.18 +/- 0.21) while AM-stimulation of cAMP levels was inhibited by CGRP (8-37) (pA2 7.41+/- 0.15) and AM (22-52) (pA2 7.26 +/- 0.18). BIBN4096BS only antagonized the effects of CGRP (pA2 8.40 +/- 0.30) on cAMP accumulation. These pharmacological profiles suggest that effects of CGRP are mediated by the CGRP1 receptor while those of AM are related to the activation of the AM1 receptor subtype. In conclusion, AM and CGRP induced c-fos expression in a similar pattern but had different effects on release of aspartate and glutamate. AM and CGRP induced cAMP accumulation in the spinal cells by their specific receptors.

O-909

EFFICIENCY AND SPECIFICITY OF ANTISENSE OLIGODEOXYNUCLEOTIDES AND SIRNA DIRECTED AGAINST EWS-FLI-1 JUNCTION ONCOGENE IN EWING SARCOMA

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Ewing sarcoma, a metastatic cancer of children and young adults, contains a translocation between EWS gene on the chromosome 22 and Fli-1 gene on the chromosome 11. Expression of EWS/fli-1 fusion gene results in a chimeric protein which has a predominant role in the transformed phenotype of Ewing sarcoma. The junction point at mRNA level offers a specific therapeutic target for antisense strategy; however, there is a strong argument in recent publications that antisense oligodeoxynucleotide (AsODN) and especially small interfering RNA (SiRNA) may control hundreds of genes. This is more notable in the case that EWS and EWS/fli-1 proteins are both active transcription factors; therefore, we aimed to determine not only efficiency but also specificity of AsODN of different design and SiRNA against EWS/fli-1 breakpoint junction in Ewing sarcoma. NIH3T3 cells were transformed by introducing EWS/fli-1 oncogene to the cells. This caused alterations in cellular morphology and the potentiation of proliferative and antiapoptotic pathways. AsODN and SiRNA were delivered to the cells by artificial nanovectors. Intracellular trafficking was studied by FACS and fluorescence microscopy. Growth inhibition was determined by MTT assay in comparison with scrambled sequences. Pattern of gene expression was studied by Real-Time RT-PCR. Inhibition of EWS/fli-1 expression, an indicator of efficiency, was studied with respect to EWS and fli-1 genes, indicators of specificity. NIH3T3/EWS-fli1 were injected subcutaneously and inoculated into nude mice to study the size of xenograft tumor in-vivo. Efficiency and specificity were sequence and backbone dependent. There was no linear correlation between the level of target gene expression and the growth inhibition. AsODN and SiRNA, which inhibit the tumor growth in-vivo, could be considered as therapeutic molecules.

p-910

EXPERIMENTAL VISCERAL LEISHMANIASIS IN BALB/C MICE CAN BE INHIBITED BY CYSTEINE PROTEINASE TYPE III

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Visceral leishmaniasis or kala-azar is a chronic systemic disease and could be fatal if not treated. Vaccination is the only tool to control it. One of the virulence factors of Leishmania infantum is Cysteine proteinase type III or CPC (belong to cathepsin B like proteinases) and can be a good candidate for vaccination. In present study, the efficacy of vaccination with cysteine proteinase type III is investigated. For this purpose, cpc gene was isolated from L. infantum genomics by PCR. After sequence confirmation, the cpc fragment was cloned in pQE40 as fused gene with DHFR and then purified using Ni-NTA. Sera reactivities of different stage of cutaneous and visceral Leishmaniasis showed that rCPC is highly immunogenic in human. Prime-boost vaccination was carried out in three groups of Balb/c mice. Test group were primed with pcDNA-cpc and boosted with rCPC protein. Control groups received pcDNA and rDHFR or PBS. Mice were challenged with 2 × L. infantum (iv). Parasite burden were measured in liver and spleen every two weeks after challenge up to fourteen weeks. Humoral immune responses (total IgG, IgG1, IgG2a) were tested before and seven weeks after challenge. Nitric oxide concentration in peritoneal macrophages measured seven weeks after challenge. Our results showed that the ratio of IgG2a/IgG1 and nitric oxide concentration is strongly higher in test group than control groups. The parasite load of test group is significantly lower than control. Our data indicate that DNA/protein vaccination with cpc can induce an acceptable TH1 response against L. infantum in BALB.

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