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RESEARCH ARTICLE

Protective effects of *Biebersteinia multifida* on sub-chronic toxicity of DZN in male Wistar rats: biochemical, hematological, and oxidative stress indices

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ABSTRACT

The protective effect of *Biebersteinia Multifida* on diazinon-induced toxicity in male Wistar rats was investigated over 8 weeks. Impacts of diazinon (10 mg/kg daily), *Biebersteinia Multifida* (500 mg/kg daily), and coadministration of them on oxidative stress parameters besides hematological and biochemical indices were assessed in various groups. The gas chromatography-mass spectrometry analysis was performed to identify the antioxidant components of plant extract by comparing the mass spectra and retention indices with those given in the literature. Pseudocholinesterase level demonstrated a significant attenuation in the *Biebersteinia Multifida*+diazinon-treated group in comparison to the diazinon group at the end of the 8th week. Statistical significant differences in hematological and biochemical indices were detectable when the diazinon group was compared to *Biebersteinia Multifida*+diazinon-treated rats. While diazinon destroyed hepatic and renal functions, *Biebersteinia Multifida* protected the liver and kidney from diazinon toxic effects by normalizing related function indices at the end of the 8th week. By diminishing malondialdehyde and enhancing the ferric-reducing power, *Biebersteinia Multifida* minimized the hazardous effect of diazinon-induced oxidative stress. Following these results, the beneficial effects of *Biebersteinia Multifida* in reducing the toxicity of diazinon should be taken into consideration.

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Biebersteinia Multifida; diazinon; oxidative stress; hematology; biochemistry; gas chromatography mass spectrometry

1. Introduction

Diazinon (DZN), the most commonly used organophosphorus (OPs) pesticide, is to be concerned about high toxicity for vertebrates, despite its low persistence in the environment (Boyda *et al*. 2021, Li *et al*. 2021).

Due to its lipophilicity, there is a hazard of delayed toxicity if significant amounts of DZN are accumulated in fatty tissues. DZN can reach all the tissues and exerts severe adverse effects. Long-term DZN exposure has been associated with immunotoxicity (Darvishi *et al*. 2022), neurotoxicity (Karimani *et al*. 2021), hepatotoxicity (Yaghubi Beklar *et al*. 2021), metabolic disorder (Nili-Ahmadabadi *et al*. 2019), inflammation (Abdel-Diam *et al*. 2019), kidney injury (Najeb *et al*. 2021), and decreased fertility (Harchegani *et al*. 2018).

DZN functions as an acetylcholinesterase inhibitor (AChEI) and will cause an abnormal accumulation of acetylcholine (AChE) in the synaptic cleft (Slotkin *et al*. 2019).

Increasing evidence suggests toxic effects of DZN following subchronic and chronic exposure mediated by the induction of oxidative stress (Tatipamula *et al*. 2022) which usually arises as a result of the conversion of DZN to diazoxon by the liver microsomal enzymes (Karimani *et al*. 2019).

Disequilibrium between oxidants and antioxidant species can lead to a distortion of biological macromolecules and it may also be extended to cause histopathological disorders (Elsayed *et al*. 2022a, 2022b). Therefore, it is logical to enhance antioxidant capacity as an effective strategy to ameliorate the chronic toxic effects of DZN (Sule *et al*. 2022).

Herbal compounds are assumed to have the capacity to modulate oxidative stress due to numerous natural antioxidants and therefore, they are believed to have beneficial effects on attenuating or averting long-term adverse effects associated with DZN exposure (Al-Attar *et al*. 2017).

Biebersteinia is a perennial herb comprising four species, namely *B. heterostemon, B. multifida* (BM), *B. odora*, and *B. orphanidis* in the flowering plant order Spindale (Yamamoto *et al*. 2014).

These species are widely distributed on the rocky slopes of semi-arid regions from the East Mediterranean to West Siberia and Central Asia (Jalilvand *et al*. 2019).

BM is stemless and has tuberous rhizomes which resemble the outline of a little fat man known as Adamak in Iran. BM has a long history as traditional folk medicine in Iran, especially among shepherds (Keshavarzi *et al*. 2018).

The ointment prepared from the root of BM has been used to treat musculoskeletal disorders and heal bone fractures (Keshavarzi *et al*. 2018). Also, BM possesses significant

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pharmaceutical effects on humans including anti-inflammatory (Farsam *et al*. 2000, Irvani *et al*. 2021), anxiolytic (Monsef-Esfahani *et al*. 2013), analgesic (Irvani *et al*. 2021), antibacterial (Zhang *et al*. 2020), and antioxidative properties (Nabavi *et al*. 2010).

Bioactive constituents isolated from leaves and roots of Bibersteinia plants include flavonoids, alkaloids, terpenoids, guanidines, sterols, and essential oils (Greenham *et al*. 2001).

To the best of our knowledge, there are no studies in the literature reporting the antioxidant effects *in vivo* of BM against subchronic-DZN toxicity. Accordingly, the objective of this study where to determine the antioxidant fractions of BM root extract through gas chromatography-mass spectrometry (GC-MS), the impact of sublethal dose of DZN on hematological, biochemical, and oxidative stress indices, effects of BM alone on the above parameters, and eventually the role of BM in attenuating DZN toxicity.

2. Materials and methods

2.1. Chemicals

Except for DZN which was a Sigma product, all other chemicals used in this study were purchased from Merck (Darmstadt, Germany).

2.2. Animals

Twenty-four adult male Wistar rats weighing around 230 g, were obtained from the animal house of Avicenna Research Institute, Mashhad, Iran and kept in there under a completely standard situation throughout the study. All animal procedures were approved by the Animal Welfare Committee of the School of Veterinary Medicine, Ferdowsi University of Mashhad, Iran, and the national laws for the experiment on animals (IR.UM.REC.1400.102).

2.3. Plant preparation

BM was collected in April 2021 from the mountains of Khorasan Province, Iran. The plant was identified by the Biology department, Ferdowsi University of Mashhad, Iran.

Gathered plants were dried in the shade and their roots were separated. The aqueous extract of BM was prepared by filtering 200 g dried root powder of BM with 2.0 L distilled water in a sterilized environment for 2 days. The dilution was shaken three times a day (100 rpm). After percolation with Whatman filter paper (No. 1) and centrifuging (4500 rpm, 8 m), the solvent was removed under vacuum at 40 °C. The remained residues were kept in a freezer at −80 °C until further tests. To administer the extract (500 mg/kg), distilled water was used as a diluent (Raeesi *et al*. 2019).

2.4. The GC/MS analysis

The GC/MS analysis was performed on an Agilent 5975C MSD mass spectrometer with a gas chromatograph with an HP 5MS column (60 m × 0.320 mm, film thickness 0.25 μm). H2 was used as a carrier gas (1.3 ml/min). The column temperature was kept at 70 °C for 2 min and programmed to reach 220 °C at the rate of 5 °C/min and stayed steady at 220 °C for 3 min. The components of plant extract were then identified by comparison of their mass spectra and retention indices (RI) with those given in the literature.

2.5. Animal treatment schedule

Rats were assigned randomly to four groups. The control group rats received 10 mg/kg corn oil, BM-treated rats were given BM 500 mg/kg BW in distilled water, DZN was administered in corn oil (10 mg/kg BW) in the DZN group, and eventually, BM + DZN-treated received BM (500 mg/kg BW in distilled water) as a pretreatment 1 hour before administration of DZN (10 mg/kg BW in corn oil). All the rats received their selected treatment once a day orally through gavage and were under complete observation throughout 8 weeks. Regarding the outcomes of previous studies, showing oxidative stress induction by DZN or having beneficial therapeutic effects in administrating BM, the selection of DZN (Karimani *et al*. 2019) and BM (Raeesi *et al*. 2019) doses was done.

2.6. Measurement of body and organ weights

Bodyweight was measured weekly throughout the experiment by automatic balance (AND GX-600, Japan). At the end of the treatment procedure, animals were euthanized by CO2 gas. Various organs were collected and cleaned immediately and weighed. The relative organ weight is expressed as wet organ weight/live body weight.

2.7. Evaluation of hematological and biochemical parameters

After euthanizing, blood samples were taken from hearts and transferred to two separate sterile tubes for further hematological and biochemical analysis. EDTA was added to one of the tubes immediately. Hematological indices in EDTA-anticoagulated blood samples were evaluated by using a Nihon Kohden Celltac Alpha hematology analyzer (Japan). In the other tube, blood was allowed to clot for 2 h at room temperature and then centrifuged at 2000 rpm for 15 min to obtain serum. The collected serum was stored at −20 °C until serum biochemistry analysis using Pars Azmoon kits in Targa 3000 autoanalyzer (Italy).

2.8. Tissue oxidative stress markers assay

2.8.1. Tissue preparation

After detaching, organs were cleaned from extraneous material, cut into small pieces, and dried on filter paper. Homogenizing was done by use of a 'Silent crusher M' type homogenizer (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) in ice-cold 1.15% KCl-0.01 M sodium, potassium phosphate buffer (pH 7.4) (10% *w/v*). The homogenate was centrifuged (18,000 g, 20 min, 4 °C) and the

supernatant was collected for oxidative stress markers determination.

2.8.2. TBARS assay

MDA, the end product of lipid peroxidation, reacts with TBA as a thiobarbituric acid reactive substance (TBARS) and produces a red-colored complex with a peak absorbance at 532 nm. Homogenized tissue was prepared for further analysis regarding the methods mentioned in our previous work. The nmol of MDA per ml was calculated using 1.56×10^5 as the extinction coefficient (Karimani *et al*. 2019).

2.8.3. Total antioxidant capacity assay

Tissue homogenates were used to evaluate total antioxidant capacity. Reducing antioxidant power (FRAP) which assays the reduction of ferric tripyridyl-s-triazine (Fe^{3+}-TPTZ) complex to ferrous tripyridyl-s-triazine (Fe^{2+}-TPTZ) has been used. Via a spectrophotometer at 593 nm, the intensive blue color of Fe^{2+}-TPTZ has been monitored for up to 5 min. The calibration curve of FeSO47H2O (100–1000 mM) has been used to set final calculations (Karimani *et al*. 2019).

2.9. Statistical analysis

The statistical analysis was performed by using SPSS 11.5 (SPSS Inc., Chicago IL, USA). Differences between groups were assessed using analysis of variance (ANOVA) followed by Tukey multiple comparisons. Data are presented as mean ± standard error of the mean (SEM). A p values <0.05 was regarded as significant.

3. Results

3.1. GC-MS analysis

The GC-MS chromatogram of the aqueous rhizome extract of BM detected a total of peaks corresponding to the bioactive compounds that were identified by relating their peak relation time (RT), and their peak area. Based on abundance, the top three major compounds present in the aqueous extract were phenol, thymol, and flavone (luteolin) (Figure 1).

3.2. Effect of DZN and BM on body and organ weight

Symptoms of acute DZN poisoning and death did not become manifest in any of the experimental groups until the end of the study. Significant changes in body weight were not identified between BM-treated and BM + DZN-treated groups compared with the control group during the experimental period. The same trends were identified in the DZN-treated group at the end of the 1st, 2nd, 3rd, 4th, and 5th weeks but at the 6th, 7th, and 8th weeks after exposure to DZN, significant weight loss was observed ($p < 0.05$). So that the biggest weight loss was recorded at the end of the study (8th week) in the DZN-treated group ($p < 0.05$) (Figure 2). DZN-treated rats revealed that DZN caused an increase in the liver and kidney weights and a decrease in the spleen weight ($p < 0.05$).

BM- and BM + DZN-treated groups were not shown any significant organ weight changes compared to the control ($p > 0.05$) (Figure 3).

3.3. Effects of DZN and BM on hematological parameters

As shown in Table 1, a significant decrease in the neutrophils and an increase in the lymphocytes appeared in the DZN group in comparison with other groups ($p < 0.05$).

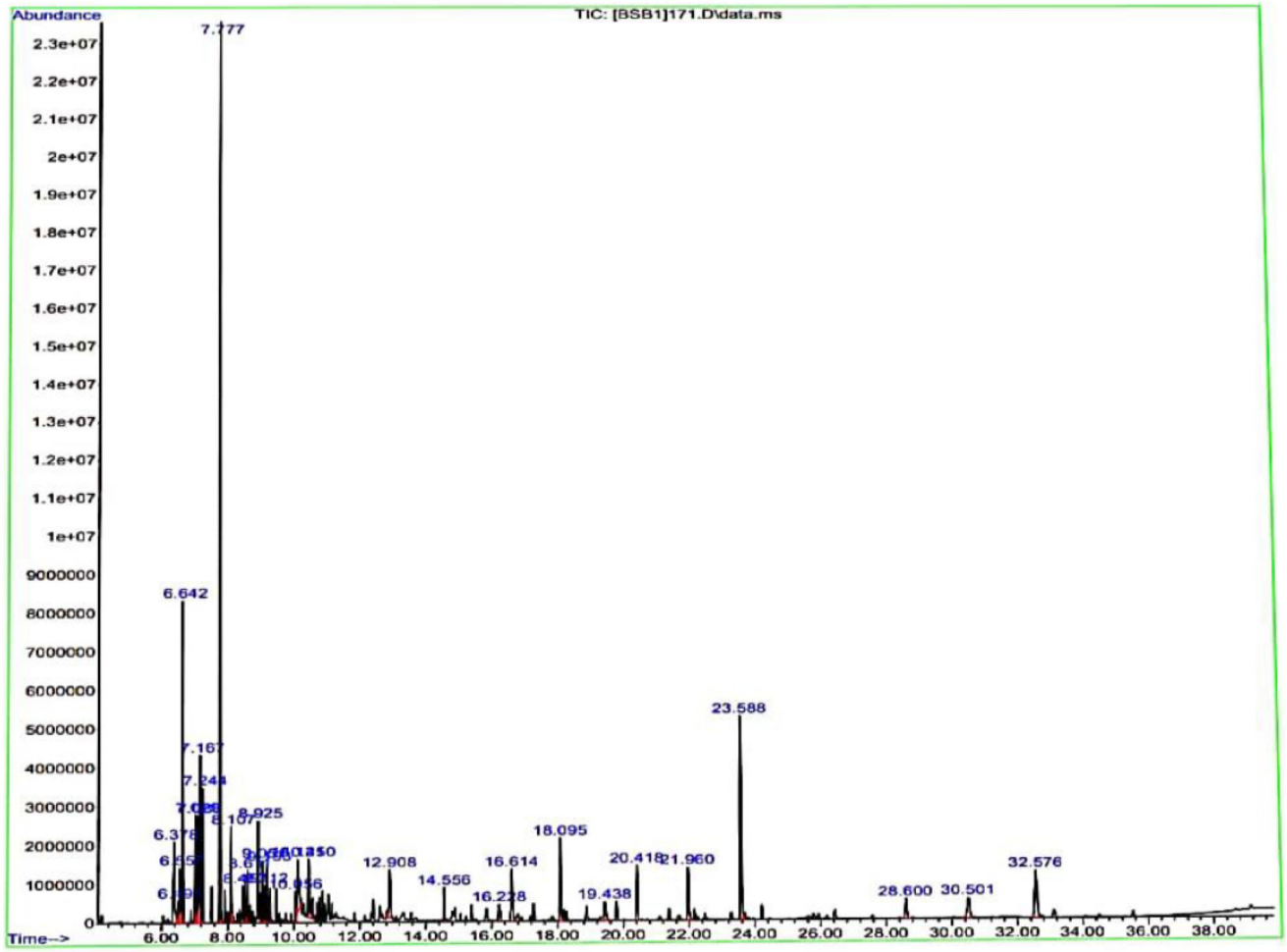
In the BM- and BM + DZN-treated groups, related leukogram changes were similar to the control one. The comparison of erythrogram including red blood cell (RBC) counts, hemoglobin, hematocrit, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and thrombocytes counts between BM-treated and control groups elaborated no significant difference. DZN reduced RBC, hemoglobin, hematocrit, and thrombocytes ($p < 0.05$) in comparison with the DZN + BM-treated group. These factors were markedly increased in DZN + BM-treated rats at the end of the 8th week. Also, DZN caused an elevation in MCV levels ($p < 0.05$). Among all of the factors, MCV and MCHC reverted to the normal range post-BM administration.

3.4. Determination of hepatic function parameters

To evaluate liver damage induced by DZN and the protective effect of BM, the activities of some hepatic enzymes were measured as hepatotoxic biomarkers. BM treatment alone did not significantly alter liver enzyme levels. In contrast, DZN administration induced liver dysfunction as revealed by high levels of liver enzymes compared to the control group ($p < 0.05$). In the BM + DZN-treated group the levels of alanine aminotransferase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) were partially recovered while aspartate aminotransferase (AST) fully recovered when compared to the DZN group ($p < 0.05$) (Table 2). There was a statistically significant increase in total bilirubin levels in the DZN-treated group ($p < 0.05$). It is noteworthy to mention that due to the subsequent subchronic DZN administration, total protein, albumin, and globulin markedly dropped ($p < 0.05$) but BM significantly modified them in BM + DZN treated rats ($p < 0.05$) (Table 2).

3.5. Effect of DZN and BM on blood sugar and lipid profile changes

DZN-induced hyperglycemia was observed at the end of the 8th week ($p < 0.05$). BM-treated rats showed a statistically significant reduction in the blood glucose concentration compared to the control ones on day 56 ($p < 0.05$). A significant decrease in blood sugar levels has been found in the BM + DZN group compared to the DZN group ($p < 0.05$) (Table 2). Meanwhile, the results demonstrated that DZN raised significantly lipid profiles including total cholesterol (TC), and total triglycerides (TG) above the normal range but BM pretreatment significantly lowered both of them



Sequence	Compound	RT	%
1	Thymol	6.64	12
2	Phenol	7.77	40
3	FLAVONE (Luteolin)	23.588	10

Figure 1. Results of *Biebersteinia Multifida* analysis.

($p < 0.05$). BM treatment significantly reduced TC and TG in comparison with control rats ($p < 0.05$) (Table 2).

3.6. Effect of DZN and BM on renal function parameters

Strong evidence of DZN-induced nephrotoxicity was found. The results demonstrated that the DZN caused a significant increase in the amounts of blood urea nitrogen (BUN), serum creatinine (Cr), and uric acid (UA) ($p < 0.05$). However, BM pretreatment significantly reduced BUN, Cr, and UA when BM + DZN treated rats were compared to DZN ones ($p < 0.05$) (Table 3). BM treatment alone did not change these parameters compared to the control group.

3.7. Effect of DZN and BM on pseudocholinesterase activity

Serum AchE activity among various groups, with DZN-treated and DZN + BM-treated rats having 41.66 and 28.12% lesser activity respectively, as compared with the control ($p < 0.05$) (Table 2).

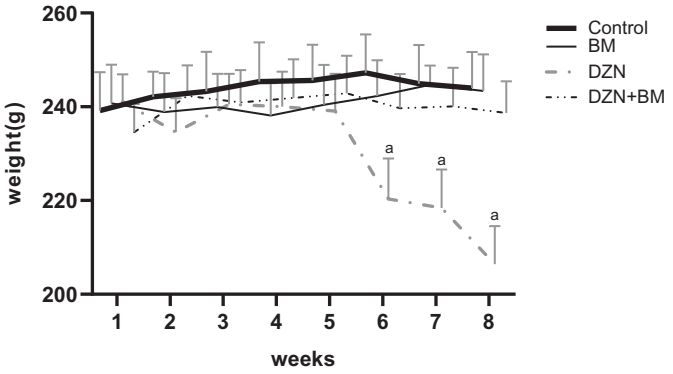


Figure 2. Effect of DZN (DZN 10 mg/kg), *Biebersteinia Multifida* (BM 500 mg/kg), and DZN plus BM (10 and 500 mg/kg), on the bodyweight alteration in rats. BM in distilled water and DZN in corn oil were given through gavage to rats for 8 weeks. Throughout 8 weeks, there was no significant change in body weight of control, BM, and DZN + BM groups. A significant bodyweight decrease was proved in DZN groups compared to the control group, in the last three weeks ($p < 0.05$). Data showed as mean ± SEM, $n = 6$. [a]Comparison with the control group.

3.8. Effect of DZN and BM on oxidative stress parameters

3.8.1. Effect of DZN and BM on lipid peroxidation

As shown in Table 3, the results indicate that DZN induced lipid peroxidation in the brain, liver, spleen, heart, kidney, and serum while BM pretreatment to DZN gavage significantly reduced serum and tissue MDA levels ($p < 0.05$) but these values did not reach reference ranges despite heart and kidney MDA levels that completely reverted ($p < 0.05$).

3.8.2. Effect of DZN and BM on total antioxidant capacity

Antioxidant status was evaluated in terms of the ferric-reducing antioxidant power in the above-mentioned tissues. DZN administration significantly depressed the FRAP values in the brain, liver, spleen, heart, and kidney ($p < 0.05$) but BM alone intensified antioxidant capacity in all tissues as compared to the control ($p < 0.05$) (Table 3). The administration of BM before DZN rebounded the FRAP levels of the spleen, heart,

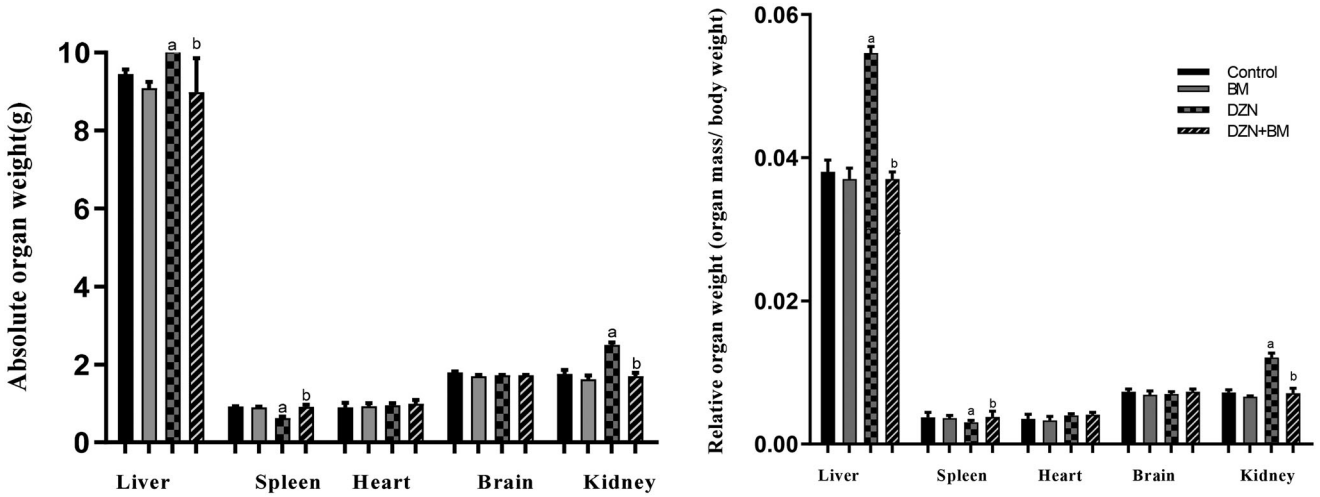


Figure 3. Effect of DZN (10 mg/kg), *Biebersteinia Multifida* (BM 500 mg/kg), and DZN plus BM (10 and 500 mg/kg) on the absolute and relative liver, spleen, heart, brain, and kidney weight in Wistar rats. This figure shows that DZN can increase the absolute weight of rat liver, spleen, and kidney compared to control groups ($p < 0.05$). Data showed as mean ± SEM, $n = 6$. [a]comparison with the control group.

Table 1. Effect of diazinon (DZN 10 mg/kg), *Biebersteinia Multifida* (BM 500 mg/kg), and DZN plus BM (10 and 500 mg/kg) on hematological parameters in the serum of Wistar rats.

Parameters/Groups	Control	BM	DZN	DZN + BM
WBC (*10^3/uL)	10.51 ± 0.90	9.98 ± 0.98	9.84 ± 1.67	9.64 ± 0.12
Neu (*10^3/uL)	2.24 ± 0.31	1.98 ± 0.38	0.9 ± 0.26[a]	2.04 ± 0.33[b]
Lym (*10^3/uL)	7.14 ± 0.51	6.89 ± 0.34	8.73 ± 0.22[a]	7.5 ± 0.09[b]
Mono (*10^3/uL)	0.23 ± 0.14	0.21 ± 0.25	0.32 ± 0.14	0.30 ± 0.52
RBC (*10^6/uL)	7.87 ± 0.42	7.45 ± 0.83	5.64 ± 0.11[a]	6.38 ± 0.1[b]
Hemoglobin (g/dL)	14.86 ± 1.02	13.70 ± 0.69	9.56 ± 0.47[a]	12.05 ± 0.38[a,b]
Hematocrit	45.1 ± 0.36	43.3 ± 0.25	31.81 ± 1.25[a]	36.5 ± 1.12[a,b]
MCH (pg)	18.86 ± 0.23	19.53 ± 0.52	19.75 ± 0.83	18.93 ± 0.42
MCV (fL)	58.62 ± 3.49	57.37 ± 2.86	68.56 ± 3.32[a]	60.33 ± 4.78[b]
MCHC (g/dL)	32.63 ± 1.56	31.98 ± 3.62	27.31 ± 2.78[a]	31.86 ± 2.63[b]
Thrombocyte (*10/μL)	338.87 ± 12.34	336.53 ± 8.24	150.31 ± 14.56[a]	220.28 ± 13.42[a,b]
AchE (U/L)	76.3 ± 3.9	75.6 ± 3.6	44.8 ± 3.7[a]	55.2 ± 0.8[a,b]

Diazinon (DZN 10 mg/kg) in corn oil, *Biebersteinia Multifida* (BM 500 mg/kg) in distilled water, and DZN plus BM (10 and 500 mg kg^{-1}) were given through gavage to rats for 8 weeks.
Data showed as mean ± SEM. $p < 0.05$, Tukey–Kramer test, $n = 6$.
[a]Comparison with the control group; [b]comparison with the DZN-treated group.

Table 2. Effect of diazinon (DZN 10 mg kg^{-1}), *Biebersteinia Multifida* (BM 500 mg kg^{-1}), and DZN plus BM (10 and 500 mg kg^{-1}) on hepatic parameters, kidney function, sugar and lipid profiles in the serum of Wistar rats.

Parameters	Control	BM	DZN	DZN + BM
Total protein (g/dL)	7.66 ± 0.49	7.35 ± 0.56	5.51 ± 0.65[a]	6.22 ± 0.22[a,b]
Albumin (g/dL)	5.47 ± 0.32	5.52 ± 0.51	3.99 ± 0.43[a]	4.43 ± 0.12[a,b]
Globulin	2.2 ± 0.03	2 ± 0.04	1.52 ± 0.06[a]	1.89 ± 0.03
Total bilirubin (mg/dL)	0.6 ± 0.05	0.58 ± 0.03	0.72 ± 0.09[a]	0.62 ± 0.03
Urea (g/dL)	39.86 ± 0.66	40.12 ± 0.34	58.87 ± 0.52[a]	49.36 ± 037[a,b]
Creatinine (g/dL)	0.73 ± 0.05	0.76 ± 0.08[a,b]	1.12 ± 0.06[a]	0.86 ± 0.03[a,b]
Uric acid (mg/dL)	1.33 ± 0.34	1.62 ± 0.51	3.96 ± 0.72[a]	2.12 ± 0.12[a,b]
Glucose (mg/dL)	92 ± 5.8	74 ± 4.5[a]	210 ± 6.6[a]	154 ± 5.3[a,b]
Cholestrol (mg/dL)	91 ± 5.13	74 ± 5.69[a]	149 ± 4.12[a]	112 ± 6.6[a,b]
Triglyceride (mg/dL)	105 ± 0.75	85 ± 0.16[a]	156 ± 1.78[a]	119 ± 1.9[a,b]
AST(U/L)	144.6 ± 6.8	138.8 ± 5.6	210.7 ± 8.7[a]	149.9 ± 5.6[b]
ALT(U/L)	27.00 ± 6.8	30.3 ± 5.5	56.6 ± 3.9[a]	41.2 ± 3.5[a,b]
ALP(U/L)	145 ± 4.8	127 ± 5.2	288 ± 6.8[a]	176 ± 5.4[a,b]
LDH(U/L)	141.00 ± 5.7	148 ± 6.5	260 ± 6.8[a]	180 ± 8.9[a,b]

Diazinon (DZN 10 mg/kg) in corn oil, *Biebersteinia Multifida* (BM 500 mg/kg) in distilled water, and DZN plus BM (10 and 500 mg kg^{-1}) were given through gavage to rats for 8 weeks.
Data showed as mean ± SEM. $p < 0.05$, Tukey–Kramer test, $n = 6$.
[a]Comparison with the control group; [b]comparison with the DZN-treated group.

and kidney to normal but not yet completely returned in the brain and liver ($p < 0.05$) (Table 3).

4. Discussion

When DZN is exposed to the body for an extended period at low doses, adverse effects may be linked to non-cholinergic mechanisms (Farkhondeh *et al*. 2020, Karimani *et al*. 2018). Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are overproduced by DZN and its oxygen metabolite diazoxon (DZX), which causes oxidative stress (Figure 4) (Jafari *et al*. 2012, Al-Attar *et al*. 2017, Danaei *et al*. 2019, Karimani *et al*. 2019, Tatipamula *et al*. 2022). Although AChE activity decreased in the DZN group compared to the control group, no signs of acute cholinergic poisoning occurred. On the contrary, BM root compounds had a relative ability to recover the AChE.

In the current study, DZN-exposed rats had less appetite than the control, which was likely brought on by inflammatory mediators that suppress appetites, such as α-TNF and β κ–NF (Karimani *et al*. 2019). In BM + DZN rats, the aqueous BM root extract had a preventative impact on weight loss. The increase in appetite can be justified by the anti-inflammatory properties of the BM (Farsam *et al*. 2000).

In the DZN-exposed rats, the increased weight of the liver and kidney may be connected to tissue edema, hepatocyte, and renal hypertrophy brought on by the induction of enzymes, peroxisome proliferation, and fat infiltration. Compared to the control group, the absolute and relative spleen exhibited a substantial decline. The fact that BM prevented liver, kidney, and spleen weight changes in the BM + DZN-treated group compared to the DZN group suggests that the BM plant is adequately protecting against harmful effects (Karimani *et al*. 2019).

Through the peroxidation of cellular macromolecules, which results in the breakdown of membrane phospholipids, protein, and DNA, oxidative stress contributes to pesticide toxicity. Free radical-mediated lipid peroxidation can have destructive consequences in the cells. Reactive intermediates such as DZX alters cell permeability and the function of membrane proteins by impairing membrane structure

Table 3. Effect of Diazinon (DZN 10 mg/kg), *Biebersteinia Multifida* (BM 500 mg/kg), and DZN plus BM (10 and 500 mg/kg) on lipid peroxidation (A) and total antioxidant capacity (B) in various tissues of Wistar rats.

A. MDA (nmol/mg tissue)	Control	BM	DZN	DZN + BM
Brain	15.43 ± 0.35	9.86 ± 0.85[a]	26.68 ± 1.12[a]	19.56 ± 1.23[a,b]
Liver	8.89 ± 0.14	5.3 ± 0.15[a]	18.66 ± 0.59[a]	11.2 ± 0.43[a,b]
Spleen	16.86 ± 0.81	12.35 ± 0.55[a]	23.88 ± 1.2[a]	19.69 ± 0.85[a,b]
Heart	9.50 ± 0.1	5.66 ± 0.5[a]	15.82 ± 1.32[a]	10.34 ± 1.22[a,b]
Kidney	10.34 ± 0.43	6.04 ± 0.81[a]	18.61 ± 1.2[a]	11.22 ± 0.85[a,b]

B. FRAP (μmolFe^{2+}/g tissue)	Control	BM	DZN	DZN + BM
Brain	1.53 ± 0.04	2.1 ± 0.08[a]	0.7 ± 0.03[a]	1.2 ± 0.09[a,b]
Liver	1.43 ± 0.12	1.94 ± 0.05	0.77 ± 0.1[a]	1.12 ± 0.05[a,b]
Spleen	1.96 ± 0.41	3.2 ± 0.18[a]	1.23 ± 0.32[a]	1.88 ± 0.31[b]
Heart	1.43 ± 0.06	1.86 ± 0.12[a]	0.76 ± 0.06[a]	1.32 ± 0.12[b]
Kidney	1.76 ± 0.14	2.2 ± 0.34[a]	0.85 ± 0.13[a]	1.68 ± 0.21[b]

Diazinon (DZN 10 mg/kg) in corn oil, *Biebersteinia Multifida* (BM 500 mg/kg) in distilled water, and DZN plus BM (10 and 500 mg kg^{-1}) were given through gavage to rats for 8 weeks.
Data showed as mean ± SEM. $p < 0.05$, Tukey–Kramer test, $n = 6$.
[a]Comparison with the control group; [b]comparison with the DZN-treated group.

(Birdane *et al*. 2022). Oxidative damage may provoke the loss of enzymatic activities and the structural integrity of enzymes and activate inflammatory processes that ultimately overwhelm endogenous antioxidant defenses and repair processes leading to initiate cell death (Elgazzar *et al*. 2022, Elsayed *et al*. 2022a, 2022b). DZX-induced lipid peroxidation has been linked to the pathogenesis of numerous liver and kidney toxicities (Cichoż-Lach and Michalak 2014; Figure 4). Marked elevation in plasma AST, ALT, ALP, and LDH found in the liver, heart, kidney, and brain are released during tissue damage which is associated with hepato- and renal toxicity (hepatocellular injuries) (Zeinali *et al*. 2018, Karimani *et al*. 2019, Yaghubi Beklar *et al*. 2021). Periportal necrosis causes biliary obstruction which ends in hyperbilirubinemia and elevated ALP (Ozer *et al*. 2008). Moreover, Abnormal renal function determinants indicate kidney damage following DZN exposure (Vahidirad *et al*. 2018, Zeinali *et al*. 2018). On the other hand, antioxidants protect the integrity of cell membranes, prevent enzyme leakage, and eliminate free radicals (Scialò *et al*. 2017, Majid *et al*. 2020).

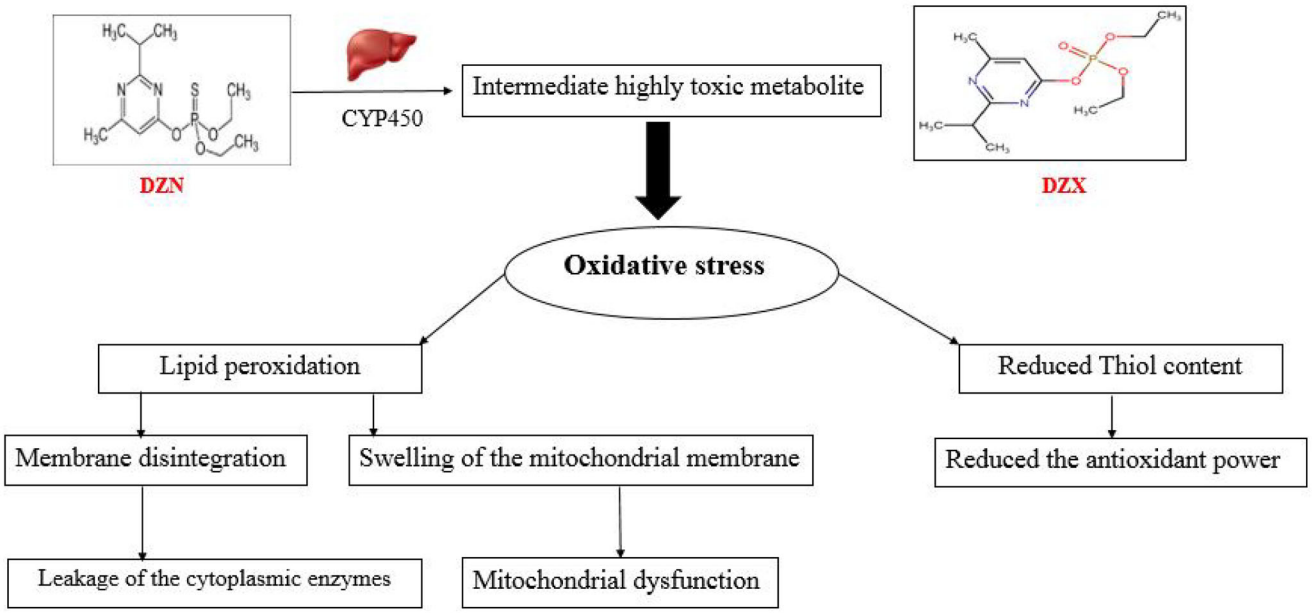


Figure 4. Diazinon cell destruction pathways.

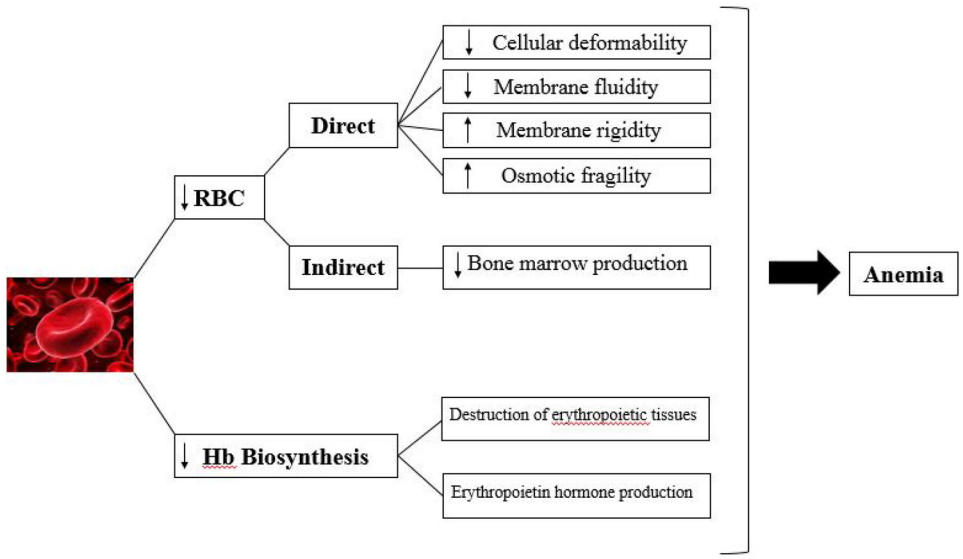


Figure 5. Mechanism of diazinon-induced hematotoxicity.

The use of antioxidants (including flavonoids) represents a rational treatment strategy to treat and prevent Hepatic injury (Kasmi *et al*. 2018).

In this study, the presence of flavonoids and phenolic rings may be responsible for BM protective effects on DZN-induced liver and kidney damage.

DZN-induced hyperglycemia is the result of damage to pancreatic islets of Langerhans, impaired insulin secretion as well as glucose production in the liver, brain, and muscles. A few studies unveil the relationship between exposure to OP insecticides and the incidence of diabetes. Therefore, OP has negative effects on insulin secretion from the islets of Langerhans, which increases blood sugar in acute and chronic exposures (Lukaszewicz-Hussain 2010).

Hypercholesterolemia can be associated with liver cell damage and cell membrane breakdown due to oxidative stress. It could be related to the reduction of hepatic lipogenesis and inhibition of pancreatic lipase activity through BM administration. According to previous studies, the consumption of BM root extract can regulate cholesterol and triglycerides (Akhlaghdoust *et al*. 2013).

In the present study, a decrease in RBC, Hb, and PCV parameters was observed in the DZN group after 8 weeks of treatment. The RBC is susceptible to lipoperoxidation changes because of its direct association with molecular oxygen, and high content of metal ions (Nita and Grzybowski 2016). DZN enhanced ROS production and decreased antioxidant levels lead to reduced erythrocyte survival and eventually hemolytic anemia (Diederich *et al*. 2018). The reduction in Hb concentration may be due to an increased rate of breakdown of red cells and/or a reduction in the rate of RBC formation (Danaei and Karami 2017). The present decrease in

HCT is attributed to the reduction in RBC count caused either due to destruction or size reduction (Samarghandian *et al*. 2020). DZN causes structural changes in RBC components under osmotic stress leading to macrocytic hypochromic anemia (Kalender *et al*. 2006). Also, DZN causes significant abnormalities in the bone marrow (Figure 5). Decreased platelet count could be a result of free radicals' effects on the bone marrow (Salehzadeh *et al*. 2019). BM root extract may also stimulate platelet production in the bone marrow or release platelets stored in the walls of blood vessels. The observed leukocytosis indicates an immune system reaction to protect the rats against infection that might have been caused by chemical and also secondary infections (Alluwaimi and Hussein 2007). Neutropenia and lymphocytosis that appeared in the DZN group were significantly improved in the BM + DZN-treated group and to the extent returned to normal. BM can modify the leukogram pattern probably by blocking inflammation associated with tissue damage. BM is an important source of iron and phenolic compounds which have antioxidant and free radical scavenging activity thus preventing the possible damage caused by the DZN (Nabavi *et al*. 2010).

5. Conclusion

The root extract of the BM plant can improve the integrity, structure, and architecture of cells in different tissues such as the liver and kidney. Therefore, BM can recover different hematological and biochemical parameters and have a good preventive effect on subchronic exposure to DZN.

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