



# Solamargine inhibits migration and invasion of human hepatocellular carcinoma cells through down-regulation of matrix metalloproteinases 2 and 9 expression and activity



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## ABSTRACT

Solamargine is a steroidal alkaloid glycoside isolated from *Solanum nigrum*. The aim of this study was to investigate the effects of solamargine on tumor migration and invasion in aggressive human hepatocellular carcinoma cells. The MTT assay was used to assess the effects of solamargine on the viability of HepG2 cells. Migration and invasion ability of HepG2 cells under solamargine treatment were examined by a wound healing migration assay and Boyden chamber assay, respectively. Western blotting assays were used to detect the expression of MMP-2 and MMP-9 proteins and MMP-2 and MMP-9 activity were analyzed by gelatin zymography assay. Solamargine reduced HepG2 cell viability in a concentration-dependent manner. At 7.5  $\mu$ M solamargine decreased cell viability by less than 20% in HepG2 cells. A wound healing migration assay and Boyden chamber invasion assay showed that solamargine significantly inhibited in vitro migration and invasion of HepG2 cells. At the highest dose, solamargine decreased cell migration and invasion by more than 70% and 72% in HepG2 cells, respectively. Western blotting and gelatin zymography results showed that solamargine reduced expression and function of MMP-2 and MMP-9 proteins. In conclusion, the results showed that solamargine significantly inhibits migration and invasion of HepG2 cells by down-regulating MMP-2 and MMP-9 expression and activity.

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## 1. Introduction

The lethal outcome of all cancers is due to the spreading of primary tumor cells and the outgrowth of secondary tumors at distant sites (Kessenbrock et al., 2010). This occurrence which is called metastases, represents the end product of the invasion-metastasis cascade, and involves dissemination of cancer cells to anatomically distant organ sites and their subsequent adaptation to foreign tissue microenvironments (Valastyan and Weinberg, 2011). The most important physiological barrier to the metastasis of tumor cells is invasion of tumor cells into the extracellular matrix (ECM) and basement membrane (BM), so the degradation of ECM and BM is one of the most important steps in the process of cancer invasion and metastasis (Steeg, 2006; Wan et al., 2013).

Matrix metalloproteinases (MMPs), also known as matrixins, are a large group of zinc-dependent proteinases responsible for

cleaving and rebuilding ECM and BM components such as collagen, elastin, gelatin and casein (Zitka et al., 2010). MMP-2 and MMP-9 play an important role in degradation of ECM and BM proteins because of their ability to destroy type IV collagen (Ruokolainen et al., 2004). In fact, MMP-2 and MMP-9 have already been shown to contribute to the establishment of metastasis-prone sites at tumor-distant organs (Kessenbrock et al., 2010). Thus, searching for novel chemotherapeutic agents targeting MMPs with high efficacy and specificity is an important objective to overcome clinical challenges.

Hepatocellular carcinoma (HCC) is the predominant histological type of primary liver cancer, accounting for 70–85% of total liver cancer (Di Bisceglie, 2009; Jemal et al., 2011; Tateishi and Omata, 2012). HCC is a common human cancer, being the fifth most prevalent tumor type and the third leading cause of cancer-related deaths worldwide (Shlomai et al., 2014). Metastasis of HCCs occurs in about 30–50% of patients, and depends upon HCC stages (Terada and Maruo, 2013). Metastatic HCC does not respond to the cytotoxic effects of most of the current chemotherapeutic agents (Lou et al., 2012). Thus, new anti-cancer agents with specific

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cytotoxicity and reduced side-effects are needed for the clinical management of HCC.

Solamargine is a steroidal glycoalkaloid isolated from plants of the Solanaceae family, such as *Solanum nigrum* L. (Fig. 1) Ding et al., 2012. Previous studies have shown that water and polyphenol extracts of *S. nigrum* attenuated migration and invasion behaviors of HCC cells (HepG2) and mouse melanoma cells (B16-F1), respectively (Wang et al., 2010; Yang et al., 2010). It has been shown that solamargine inhibits the growth of human tumor cell lines such as colon (HT-29 and HCT-15), prostate (LNCaP and PC-3), breast (T47D and MDA-MB-231), human hepatoma (PLC/PRF/5) and JTC-26 cell lines (Liu et al., 2004), although anti-migration and anti-invasion effects of solamargine on cancer cells remain unknown. Therefore, in this study we aimed to evaluate the inhibitory effects of solamargine on HCC cell migration and invasion in view of its molecular mechanism through determination of the expression and function of MMP-2 and MMP-9 proteins.

## 2. Materials and methods

### 2.1. Test drug

Solamargine was purchased from Glycomix Ltd. (Whiteknights, United Kingdom) and dissolved in DMSO (Dimethyl sulfoxide) and PBS to a final concentration of 870  $\mu\text{M}$  and stored at  $-20^\circ\text{C}$ . The drugs were freshly diluted to their final concentration in culture medium prior to the start of the experiment.

### 2.2. Reagents

Roswell Park Memorial Institute medium (RPMI 1640) and fetal bovine serum (FBS) were purchased from Gibco Industries Inc. (Vienna, Austria). The anti-MMP-2 and anti-MMP-9 antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, US). Anti- $\beta$ -actin antibody, DMSO, trypan blue and triton X-100, pyruvate, penicillin G and streptomycin were obtained from Sigma Co. (Steinheim, Germany). HRP-conjugated secondary antibody was purchased from Abcam Company (Boston, US). Microcentrifuge concentrators were obtained from Millipore (Hessen, Germany). Matrigel was purchased from BD Biosciences (California, USA). 6-well invasion chambers were purchased from SPL life Science (Pocheon-si, South Korea).

### 2.3. Cell culture

The human hepatocellular carcinoma cell line HepG2 was cultured in RPMI 1640 supplemented with 2 mM L-glutamine, heat inactivated FBS 10% (v/v), 2 g/L sodium bicarbonate, penicillin (100 U/mL), and streptomycin (100  $\mu\text{g}/\text{mL}$ ) at  $37^\circ\text{C}$  in a humidified atmosphere containing 5%  $\text{CO}_2$ .

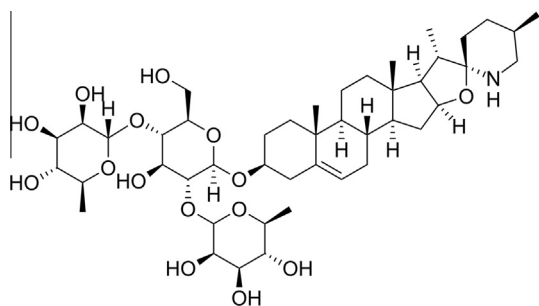


Fig. 1. Chemical structure of solamargine (Sun et al., 2010).

### 2.4. Cytotoxicity assay

The cytotoxic effect of solamargine was evaluated by the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) colorimetric assay (Kalalinia et al., 2011). Briefly, HepG2 cells were seeded in 96-well culture plates at a density of  $10^4$  cells per well. After 24 h of incubation, the medium in the 96-well plate was replaced with 100  $\mu\text{L}$  of new medium containing various concentrations of solamargine (0–7.5  $\mu\text{M}$ ) and incubated for 24 h. Control wells contained DMSO at equal volumes to those used for the test compounds. At the end of the incubation, medium was replaced with 100  $\mu\text{L}$  of MTT solution (0.5 mg/ml) and the plates were incubated for 4 h at  $37^\circ\text{C}$ . The purple MTT formazan crystals were dissolved in 100  $\mu\text{L}$  DMSO and absorbance was determined on an ELISA plate reader (BioTek, Bad Friedrichshall, Germany) with a test wavelength of 570 nm and a reference wavelength of 630 nm to obtain the sample signal. Each experiment was repeated independently 3 times in or in triplicate (MTT assay).

### 2.5. Migration assay

The anti-migration effect of solamargine on HepG2 cells was measured by a wound healing migration assay in two ways: pretreatment and treatment as described by Valster et al. (2005). For the treatment wound healing migration assay, HepG2 cells ( $5 \times 10^5$ ) were grown to 90% confluence in a 6-well plate at  $37^\circ\text{C}$ , 5%  $\text{CO}_2$  incubator. The monolayers were scratched with a yellow micropipette tip (100  $\mu\text{M}$  micropipette tip), washed with PBS to remove floating cell debris, and then incubated in the medium containing 0–7.5  $\mu\text{M}$  of solamargine for 24 h. For the pretreatment wound healing migration assay, HepG2 cells ( $4 \times 10^5$ ) were plated in 6-well plates for 24 h. Cells were then incubated with various concentrations of solamargine (0–7.5  $\mu\text{M}$ ) for 24 h. Wounds were then made similar to the methods described above and cells were incubated in the medium for 24 h. Finally, after photography, wounds sizes were measured using ImageJ software, version 1.46r.

### 2.6. Invasion assay

The effect of solamargine on the invasiveness of HepG2 cells was determined using a modified Boyden chamber technique with matrigel-coated membranes. Briefly, 6-well transwell inserts with 8  $\mu\text{m}$  polycarbonate pore size filters were coated with a uniform layer of Matrigel Basement Membrane Matrix (3 mg/ml) at  $37^\circ\text{C}$  for 1 h. The HepG2 cells ( $4 \times 10^5$  cells/1.5 ml RPMI-1640) were then placed onto the upper compartment and incubated with solamargine (0–7.5  $\mu\text{M}$ ) and the lower chamber containing 10% FBS as a chemoattractant. The plates were then incubated at  $37^\circ\text{C}$  for 24 h. Afterward MTT solution was added to each upper and lower well for an additional 3 h. The purple MTT formazan crystals were dissolved in 100  $\mu\text{L}$  DMSO and absorbance was determined on an ELISA plate reader (BioTek, Bad Friedrichshall, Germany) with a test wavelength of 570 nm and a reference wavelength of 630 nm to obtain the sample signal. Finally, the numbers of invaded cells on the bottom of each insert were measured by MTT assays using a standard curve prepared from serial dilutions of the corresponding cell line (Mansouri et al., 2014).

### 2.7. Western blot analysis

The association of the expression of MMP-2 and MMP-9 with mode of tumor invasion and metastasis involvement has previously been shown in cancer cells (Kim et al., 2013). HepG2 cells ( $4 \times 10^5$ ) were plated in 6-well plates for 24 h. Then HepG2 cells were incubated with various concentrations of solamargine (0–

7.5  $\mu\text{M}$ ) for 24 h. Afterward, for Western blot analysis of ProMMP-2 and ProMMP-9, cells were washed, harvested and lysed in Nonidet-P40 (NP-40) lysis buffer (20 mM Tris HCl pH 8, 137 mM NaCl, 10% glycerol, 1% nonidet P-40, 2 mM EDTA and cocktail proteinase inhibitor). Then, samples were homogenized by sonication and centrifuged at 14,000 rpm for 20 min and the supernatants were collected. On the other hand, for MMP-2 and MMP-9 expression analysis, 10 ml of cell culture supernatants was concentrated 100-fold in microcentrifuge concentrators. Total protein in the samples was quantitated by the BCA protein assay kit. Cell lysate (100  $\mu\text{g}$ ) or supernatant proteins (20  $\mu\text{g}$ ) were separated by 10% SDS-PAGE at 110 V, using precooled electrophoresis running buffer containing 25 mM Tris-HCl/5, 190 mM glycine, 0.1% SDS, pH 8.0 at room temperature for 60 min or until the dye front reached the bottom of the gel. Following transfer to PVDF membrane and blocking of the membranes with 2% (w/v) BSA (bovine serum albumin), blots were probed with specific primary antibodies to ProMMP-2 and ProMMP-9 (diluted 1:500) or MMP-2 and MMP-9 (diluted 1:300) or anti- $\beta$ -actin (diluted 1:5000) in 2% (w/v) BSA in PBS overnight at 4 °C. After a 15 min wash in PBS, the membranes were incubated with horseradish-peroxidase-conjugated anti-mouse secondary antibody (diluted 1:10,000) for 1 h followed by a 15 min wash in PBS. The bands were visualized using the enhanced chemiluminescence (ECL) system (Syngene, UK). Band densities were analyzed using the ImageJ software, version 1.46r, and were normalized using the density of  $\beta$ -actin bands.

### 2.8. Gelatin zymography

The activity of MMP-2 and MMP-9 in cell culture medium was measured by gelatin zymography protease assay as described by Frankowski et al. (2012). Cells were cultured ( $6 \times 10^5$  cells/ml) in 6-well plates in the presence of various concentrations of solamargine (0–7.5  $\mu\text{M}$ ) for 24 h at 37 °C in a humidified atmosphere with 5%  $\text{CO}_2$ . Supernatant aliquots (17  $\mu\text{L}$ ) of culture medium was mixed with Laemmli's sample buffer (9  $\mu\text{L}$ ) in the absence of  $\beta$ -mercaptoethanol. Samples were then loaded onto 8% sodium dodecyl sulfate polyacrylamide gels containing 1 mg/ml gelatin as a substrate. Electrophoresis was carried out at 110 V, using precooled electrophoresis running buffer containing 25 mM Tris-HCl/5, 190 mM glycine, 0.1% SDS, pH 8.0 at 4 °C for 60 min or until the dye front reached the bottom of the gel. After electrophoresis, the gel was washed with 2% (v/v) Triton X-100 for 45 min and then incubated in the developing buffer (pH 7.4) containing 50 mM Tris, 10 mM  $\text{CaCl}_2$  and 0.02%  $\text{NaN}_3$  for 42 h at 37 °C. Gels were stained with 0.05% Coomassie brilliant blue R-250 for 1 h and then the bands were visualized by destaining the gel with 30% methanol and 10% glacial acetic acid. The proteolytic activity of each sample was identified as a clear band on a blue background using ImageJ software, version 1.46r.

### 2.9. Statistical analysis

Experimental values were expressed as the mean  $\pm$  SD by IBM SPSS Statistics 19. One-way ANOVA assay and Tukey test were used to evaluate the significance of differences between groups with statistical significance considered as \* $p < 0.05$ , \*\* $p < 0.01$  or \*\*\* $p < 0.001$ .

## 3. Results

### 3.1. The effects of solamargine on HepG2 cells viability

It has been reported that solamargine shows cytotoxic effects in various cancer cell lines (Liu et al., 2004). To exclude the nontoxic concentration of solamargine on HepG2 cells which was needed in

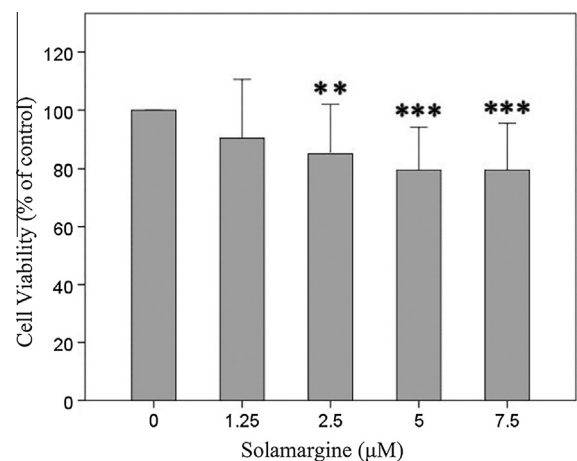
our model, HepG2 cells were treated with various concentrations of solamargine (1–7.5  $\mu\text{M}$ ) for 24 h. Then an MTT assay was performed to determine cell viability. No cytotoxicity was observed in cells treated with 1.25  $\mu\text{M}$  solamargine, whereas significantly reduced viability was found in cells treated with 2.5–7.5  $\mu\text{M}$  solamargine (cell viability compared to control, 85–79.56%, respectively) (Fig. 2). These results imply that solamargine with 0–7.5  $\mu\text{M}$  concentration, decrease cell viability of HepG2 cells by less than 50 percent. Therefore, these concentrations were selected for further evaluation of the anti-invasion and anti-migration effects of solamargine.

### 3.2. Solamargine inhibits in vitro migration of HepG2 cells

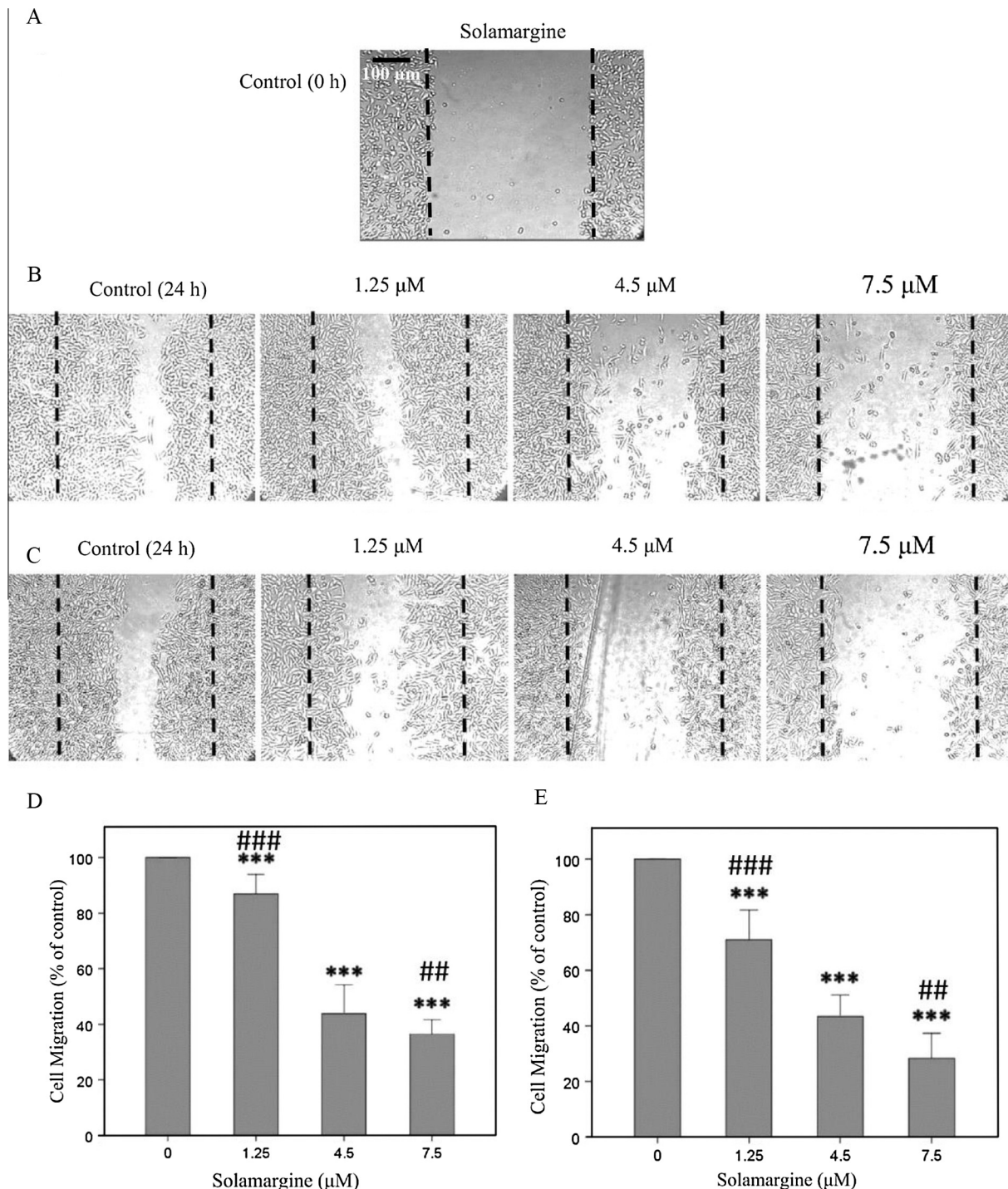
The HCC cell line HepG2 is known to have migration and invasive abilities (Yuxian et al., 2009). Therefore, the anti-migratory effects of solamargine was evaluated on the HepG2 cells using the wound healing assay which is a classical and commonly used method for studying cell migration (Yarrow et al., 2004). As shown in Fig. 3B and C, the untreated HepG2 cells exhibited a remarkable wound closure activity within 24 h incubation. Pretreatment and treatment with various concentrations of solamargine (0–7.5  $\mu\text{M}$ ) for 24 h significantly inhibited the cell migration of HepG2 (Fig. 3B and C, respectively) in a concentration dependent manner. Compared with the control, pretreatment and treatment with solamargine at 7.5  $\mu\text{M}$  concentration displayed a significant decrease in wound closures by 63.5% and 71.67%, respectively (Fig. 3D and E). The result revealed that the treatment of solamargine could diminish the migration of HepG2 cells more than pretreatment of solamargine (Fig. 3).

### 3.3. Solamargine attenuates in vitro invasion of HepG2 cells

Invasive abilities of HepG2 cells were demonstrated by cell penetration through a matrigel-coated transwell in an in vitro invasion assay. Fig. 4 shows that HepG2 cells migrated from the upper to the lower chamber in the control group (absence of solamargine) while the HepG2 cells penetration through the matrigel-coated filter was inhibited in the presence of solamargine. Exposure of HepG2 cells to 7.5  $\mu\text{M}$  solamargine reduced cell invasion activity by 67.67% compared to the non-solamargine exposed controls (Fig. 4). These results suggest that solamargine is effective in reducing HepG2 cell invasion in a concentration-dependent manner.



**Fig. 2.** The effects of solamargine on HepG2 cell viability. HepG2 cells were incubated with various concentrations of solamargine at 37 °C for 24 h. Cell viability was measured by the MTT assay. Each experiment was repeated independently 3 times in or in triplicate (MTT assay). The data are shown as the means  $\pm$  SD from three independent experiments (\*\* $p < 0.01$  and \*\*\* $p < 0.001$ ).

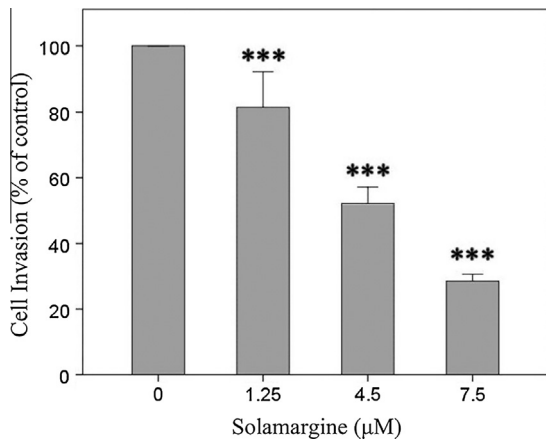


**Fig. 3.** The effects of solamargine on migration of HepG2 cells. (A) A photomicrograph of a 0 h control culture. Cells were plated and pretreated (B) or treated (C) with 0–7.5  $\mu\text{M}$  of solamargine at 37  $^{\circ}\text{C}$  for 24 h. Migration was measured by the wound healing migration assay. Finally, the closure of wounded area was calculated using ImageJ software, version 1.46r. Migration of HepG2 cells pretreated (D) and treated (E) with solamargine was blocked in a dose-dependent manner compared with that of the untreated cells. The data are expressed as mean  $\pm$  SD from three independent experiments; \*\*\* $p$  < 0.001 compared with the control. Different effects of pretreated and treated (D and E) solamargine on migration of HepG2 cells was evaluated using the Student's  $t$  test; ## $p$  < 0.01 and ### $p$  < 0.001.

#### 3.4. Solamargine reduced MMP-2 and MMP-9 proteins expression in HepG2 cells

Degradation of ECM proteins is an essential step in invasion and metastasis of cancer cells and is mainly mediated by MMPs such as MMP-2 and MMP-9 (Guilford et al., 1998; El Rifai and Powell, 2002). To test whether solamargine suppresses cancer cell invasion and motility by affecting the expression of matrix

metalloproteinase, the protein expression levels of MMP-2 and MMP-9 in the medium and the whole cell extract determined using Western blot analysis. In this regard, ProMMP-2 and ProMMP-9 levels in the cytosol (inactive form, 72 and 92 kDa, respectively) were measured and showed significant decreases of around 66% and 79% in the presence of 7.5  $\mu\text{M}$  solamargine, respectively (Fig. 5). Moreover, as compared to the control, expression of MMP-2 and MMP-9 levels in the culture medium (active

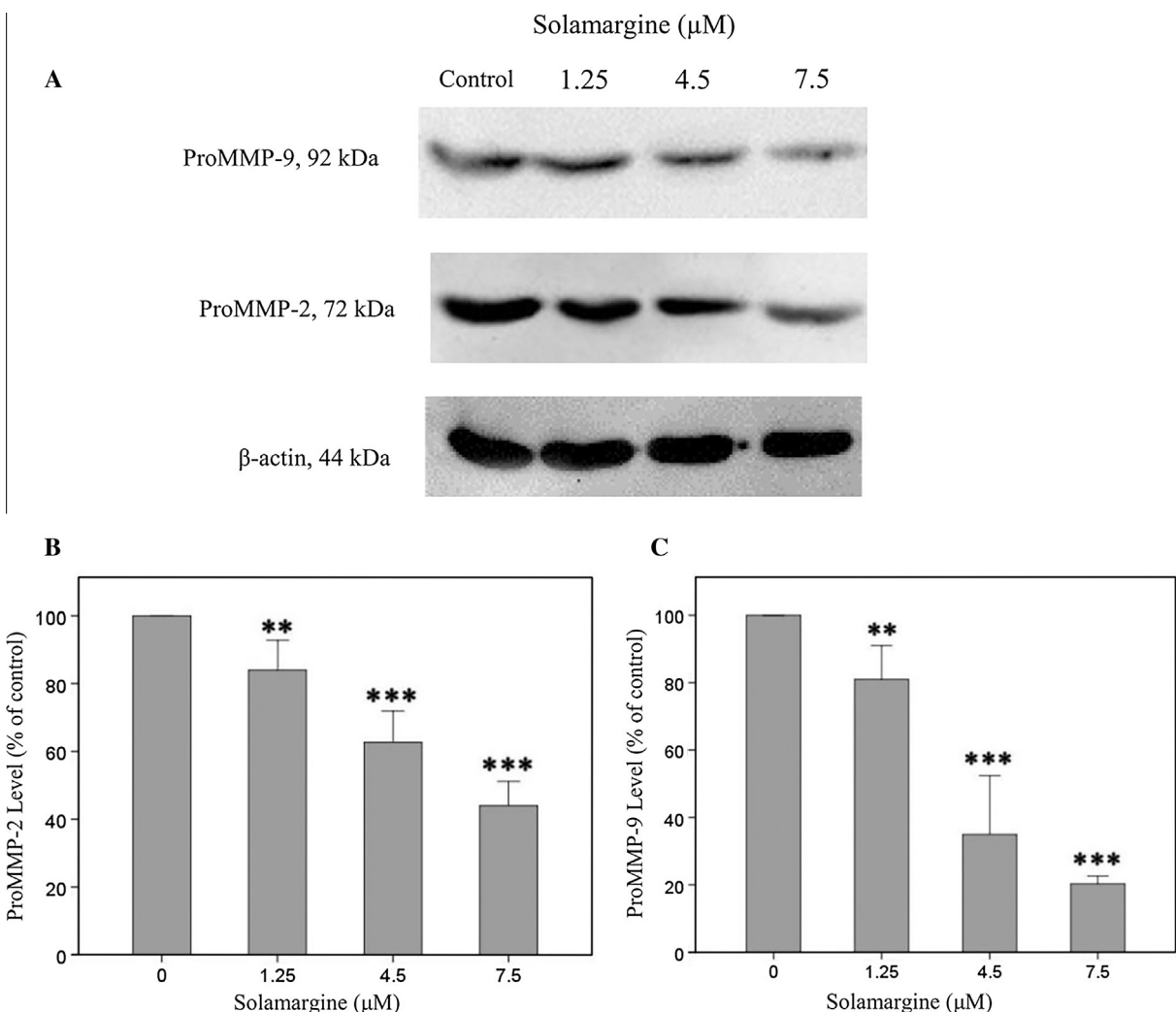


**Fig. 4.** The effects of solamargine on HepG2 cells invasion. HepG2 cells were seeded onto the upper Boyden chamber with 8 µm porous filter coated with matrigel. The lower chamber contained 10% FBS as a chemoattractant. After 24 h of incubation with 0–7.5 µM solamargine, the number of invasive cells were determined by the MTT assay. The data are expressed as mean ± SD from three independent experiments; \*\*\* $p$  < 0.001 compared with the control.

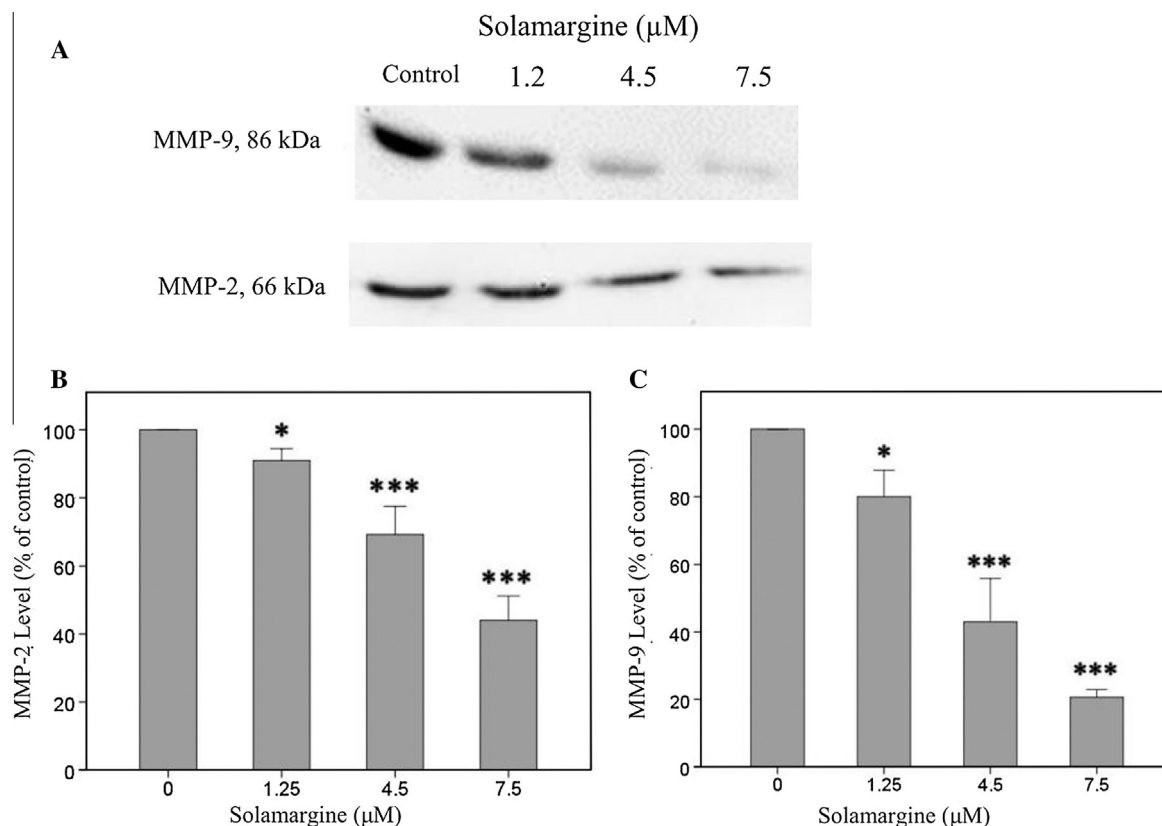
form, 66 and 86 kDa, respectively) significantly decreased after treatment with solamargine at final concentrations of 7.5 µM (64.33% and 78.5%, respectively) (Fig. 6). Results showed that treatment with solamargine for 24 h significantly decreased MMP-2 and MMP-9 expression in cytosol and culture medium of HepG2 cells in a concentration-dependent manner.

### 3.5. Solamargine reduced MMP-2 and MMP-9 enzymes activity in HepG2 cells

To investigate the alteration of MMPs activities in solamargine treated HepG2 cells, gelatinolytic activities of MMP-2 and MMP-9 were examined by a gelatin zymography assay. The mean activity of MMP-2 and MMP-9 were reduced by 37.67% and 63.67%, respectively, after treatment with 4.5 µM solamargine and by 44% and 75%, respectively, after treatment with 7.5 µM solamargine, as compared to control (Fig. 7). These results showed that solamargine reduced the enzymatic activity of the MMP-2 and MMP-9 proteins secreted from HepG2 cells in a concentration-dependent manner.



**Fig. 5.** The effects of solamargine on ProMMP-2 and ProMMP-9 protein expression. (A) HepG2 cells were incubated with 0–7.5 µM solamargine for 24 h. Cells were lysed and subjected to analysis by Western blotting. Band density was analyzed using ImageJ software, version 1.46r, and expression levels were normalized to β-actin protein level. The quantification of ProMMP-2 (B) and ProMMP-9 (C) expression is presented as mean ± SD from at least three independent experiments; \*\* $p$  < 0.01 and \*\*\* $p$  < 0.001 compared with the control.



**Fig. 6.** The effects of solamargine on MMP-2 and MMP-9 protein expression in HepG2 cells. (A) HepG2 cells were incubated with 0–7.5 μM solamargine for 24 h. Supernatants were subjected to analysis by Western blotting with anti-MMPs antibodies as indicated. The quantification of MMP-2 (B) and MMP-9 (C) protein expression is presented as mean ± SD from at least three independent experiments; \* $p < 0.05$  and \*\*\* $p < 0.001$  compared with the control.

#### 4. Discussion

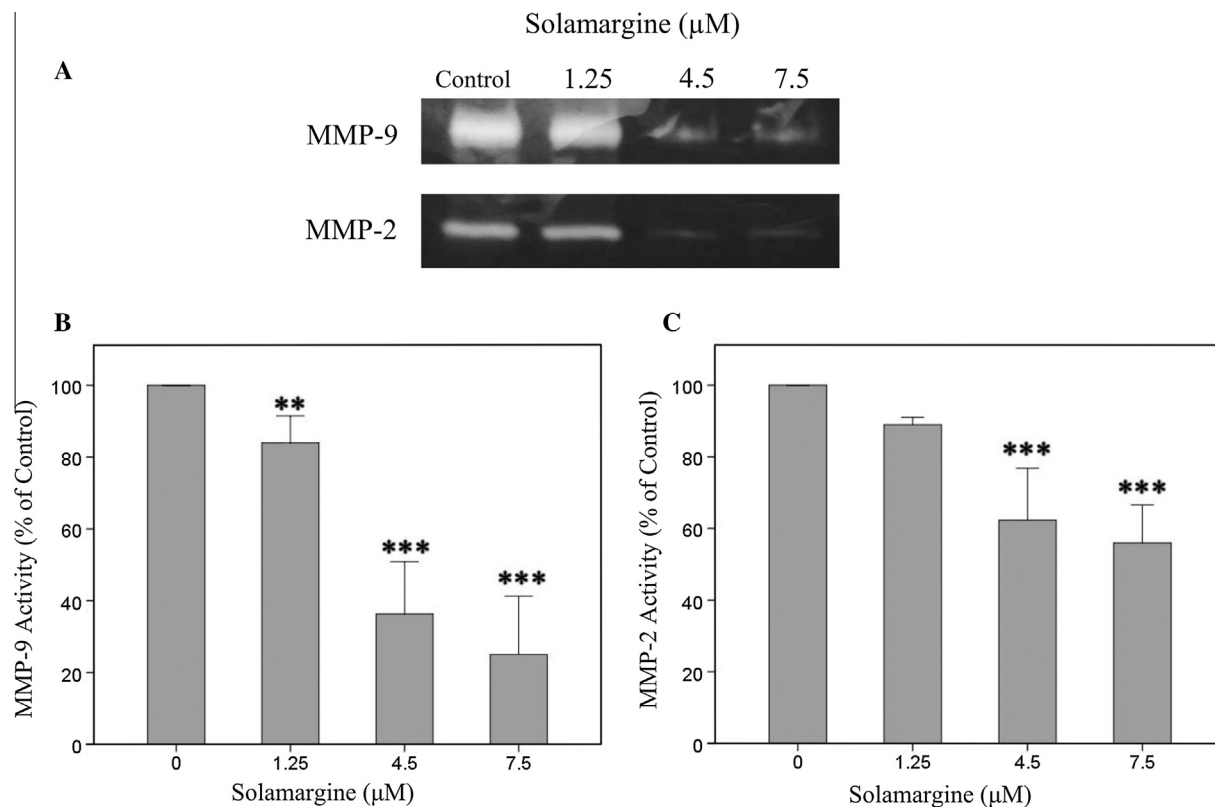
While surgical resection and chemotherapy can cure restricted primary tumors, metastatic disease is largely incurable because of its systemic nature and the resistance of spreading tumor cells to existing therapeutic agents. A mortality rate of greater than 90% has been observed in metastatic cancer rather than the primary tumors from which the malignant lesions arise (Gupta and Massague, 2006). Plant-based agents have played an important role in the treatment of cancers, and a number of them are known to inhibit metastatic progression of cancer cells (Saklani and Kutty, 2008; Meadows, 2012). *S. nigrum* is one of these plants which has attracted much attention recently because of its proven antitumor (Son et al., 2003; Li et al., 2008) and hepatoprotective effect (Hsieh et al., 2008; Lin et al., 2008). Previous studies have shown that water and polyphenol-rich extracts of *S. nigrum* attenuated migration and invasion of HCC cells (HepG2) and mouse melanoma cells (B16-F1), respectively (Wang et al., 2010; Yang et al., 2010). Solamargine, one of the main constituents of *S. nigrum* extracts, is a type of steroidal alkaloid glycoside. Although different anti-cancer effects of solamargine have been well documented (Liu et al., 2004; Li et al., 2011), so far there has been no report on anti-invasion ability of solamargine. Therefore, in the present study, we examined the effect of solamargine on invasion and migration activities of cancer cells and MMP-2 and MMP-9 expression and activity.

During the last decade, various studies have described cytotoxicity effects of solamargine in several carcinoma cell lines (Li et al., 2011; Sun et al., 2010; Shiu et al., 2007; Liang et al., 2004). In this study, the results demonstrated that solamargine decreased the percentage of HepG2 viable cells in a dose-dependent manner,

which is in agreement with previous studies. Non-toxic concentrations were selected for evaluation of the anti-invasion and migration effect of solamargine.

Tumor cell migration and invasion are key steps in tumor metastasis (Kuang et al., 2011). Previous studies showed that polyphenol and water extracts from *S. nigrum* attenuated migration and invasion of cancer cells (Wang et al., 2010; Yang et al., 2010). The effects of solamargine, a purified compound from *S. nigrum* herb, on cell migration and invasion were assessed by a wound healing migration assay and Boyden chamber invasion assay, respectively. In order to adapt the Boyden chamber migration assay to other applications such as quantitating the invasive potential of tumor cells, various extracellular matrix molecules can be coated onto the membrane. These coating materials can be laminin, collagen, natural basement membrane, or the most widely used substance known as Matrigel™ (BD Biosciences). The porous membrane is occluded by Matrigel, therefore mimicking the extracellular environment. Only invasive cells that have digested the gel will reach the underside of the membrane. The results indicated that treatment with solamargine significantly inhibited HepG2 cell migration (Fig. 3) and invasion (Fig. 4) at concentrations equal to and greater than 1.25 μM.

Metastases represent the end-products of the invasion-metastasis cascade, which involves migration of cancer cells to secondary organ sites and their subsequent adaptation to foreign tissue microenvironments (Valastyan and Weinberg, 2011). The first step in the invasion-metastasis cascade involves the degradation of the BM which is composed of type IV collagen, laminins, proteoglycans, etc. (Fidler, 2003). Degradation of the BM barrier allows direct invasion of tumor cells through the stromal compartment. Degradation of type IV collagen is effected principally by



**Fig. 7.** The effects of solamargine on MMP-2 and MMP-9 activity. (A) HepG2 cells were treated with or without various concentrations of solamargine (0–7.5 μM) for 24 h. Then MMP-2 and MMP-9 activity was analyzed by gelatin zymography. Equal aliquots of media were electrophoresed on gels (containing 1% gelatin). The effect of various concentrations of solamargine on the activity of MMP-9 (B) and MMP-2 (C) were determined using ImageJ software, version 1.46r. The data are expressed as mean ± SD from three independent experiments; \*\**p* < 0.01 and \*\*\**p* < 0.001 compared with the control.

MMPs, especially MMP-2 and MMP-9 (Kessenbrock et al., 2010; Ruokolainen et al., 2004). MMP-2 and MMP-9 are considered to be particularly good targets for anticancer drugs (Park et al., 2005). In normal tissue, the activity of MMPs is carefully controlled via transcriptional and posttranslational mechanisms (Kessenbrock et al., 2010). In this study MMP-2 and MMP-9 levels were detected with two different methods, Western blot analysis (expression) and gelatin zymography (activity). Western blotting showed that expression of MMP-2 and MMP-9 proteins in the cytoplasm and supernatant of HepG2 cells were downregulated by solamargine in a concentration-dependent manner (Figs. 5–7). Similarly, the results of the gelatin zymography showed that solamargine could reduce the activation of MMP-2 and MMP-9 secreted by HepG2 cells. In this study, the activity of MMP-9 was more clearly suppressed than that of MMP-2. Thus, in vitro evidence demonstrated that solamargine significantly inhibited invasion of HepG2 cells, at least partly by downregulating the protein expression and activation of MMP-2 and MMP-9.

It has been reported that *S. nigrum* water extract inhibits metastasis in mouse melanoma cells in vitro and in vivo. This was associated with reduced serum MMPs, Akt activity as well as Protein kinase C alpha (PKCα) and Nuclear factor NF-kappa-B (NF-κB) protein expressions (Wang et al., 2010). In another investigation, polyphenol-rich extracts of *S. nigrum* has even been reported to display anti-invasion effects on HCC cells through regulation of PKCα gene expression (Yang et al., 2010). Previous studies have demonstrated that activation of PKCα increased tumor cell invasion and metastasis, leading to the activation of the MAPKs and PI3K/Akt pathway (Lahn and Sundell, 2004; Wu et al., 2008; Barragan et al., 2002). MAPKs are serine/threonine kinases that are present in most cell types and can regulate MMP gene expressions (Chakraborti et al., 2003). In addition, the PI3K/Akt pathway

has also been reported to activate MMP expressions (Thant et al., 2000). Some key transcription-binding sites such as NF-κB are also known to be involved in the regulation of MMP gene expression (Vincenti and Brinckerhoff, 2007).

In conclusion, it was demonstrated that solamargine suppresses the invasive capacity of HepG2 cells by downregulation of MMP-2 and MMP-9 expression and activity. Therefore, these findings strongly suggest that the anti-invasive and anti-migratory properties of solamargine should be further investigated as a candidate anti-cancer drug using appropriate in vitro and in vivo models.

#### Conflicts of Interest

The authors declare that there are no conflicts of interest.

#### Transparency Document

The Transparency document associated with this article can be found in the online version.

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