Protective effects of gomisin A isolated from *Schisandra chinensis* against CCl_4-induced hepatic and renal injury

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Abstract. The aim of the present study was to investigate the protective effects of gomisin A, a lignan compound isolated from *Schisandra chinensis,* against liver and kidney damage induced by CCl_4 exposure. We assessed alterations in organ weights, levels of serum biochemical indicators, and activation of the caspase-3 and MAPK signaling pathways and carried out histological analysis of liver and kidney tissue in rats pretreated with gomisin A for four days. In the gomisin A/CCl_4-treated group, only the liver experienced a significant increase in weight, whereas the other organs did not undergo any changes. Five biochemical indicators in serum indicated that liver and kidney toxicity dramatically decreased upon gomisin A pretreatment, although the decrease in ratios varied. Upon histological analysis, the gomisin A/CCl_4-treated group showed less hepatocellular necrosis, a poorly dilated central vein in the liver section, decreased diameter of the glomerulus, a lower number of capillaries, and a convoluted tubule in the kidney section. Furthermore, the formation of active caspase-3 was inhibited by gomisin A pretreatment in the gomisin A/CCl_4-treated group, whereas the expression level of Bax protein was slightly increased. Western blot analysis revealed that there were differences between the liver and kidney in terms of activation of the MAPK signaling pathway. In the liver, gomisin A pretreatment increased phosphorylation of three members of the MAPK pathway when compared to that in the vehicle pretreatment group. However, in the kidney, only the phosphorylation level of p38 was elevated upon gomisin A pretreatment, whereas levels of the other two members were decreased. These results suggest that gomisin A induces marked protective effects against hepatic and renal injury induced by CCl_4 exposure through differential regulation of the MAPK signal transduction pathway.

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Introduction

CCl_4, often used to induce liver damage, is a well-known chlorinated hydrocarbon utilized as a solvent in various industries as well as a vermifuge in medicine to treat hookworm disease (1). For this reason, workers are often poisoned by inhalation, ingestion and absorption of CCl_4. CCl_4 also induces hepatotoxicity, nephrotoxicity and hematotoxicity (2). The liver is a major target of human CCl_4 poisoning, whereas the kidney and erythrocytes are minor target organs (3). In the liver, CCl_4 induces hepatic damage, necrosis (4) and apoptosis (5), and exposure for long periods leads to fibrosis, cirrhosis and hepatic carcinoma (6). The kidney, as a minor target, shows increased organ weight, localized glomerulosclerosis, and a higher urinary protein content in animals following exposure to a high concentration of CCl_4. To date, extensive research has been carried out in Oriental medicine to develop a novel therapeutic drug capable of preventing organ damage induced by CCl_4.

Many lignan compounds isolated from *Schisandra chinensis* have been considered as candidate substances for protection against CCl_4-induced damage. Over the past 20 years, *S. chinensis* has been well reported in traditional Chinese medicine (7), and it has been shown to contain many active lignans, including gomisins A, B, C, D, E, F, G, K3, N, J, Schisandrol B, Schisandrin and Schisandrin C (7,8). Among these, gomisin A was first reported as one of five new dibenzocyclooctadiene lignans isolated from the petroleum ether extract of *S. chinensis* fruits (9). Its structure and function have been investigated using chemical and spectral techniques in both *in vitro* and *in vivo* studies. Among the functions of gomisin A, its protective and regenerative effects against experimental liver damage induced by various factors have been well confirmed. For instance, disappearance of plasma indocyanine green (ICG) induced by CCl_4, d-galactosamine,

and orotic acid was not delayed by gomisin A, which possesses a liver function-facilitating property in normal and liver-damaged rats (10). Moreover, the development of acute hepatic failure induced by intravenous administration of heat-killed *Propionibacterium acnes* followed by a small amount of gram-negative lipopolysaccharide (LPS) for seven days was significantly protected against by ingestion of food containing 0.06% gomisin A for 4 weeks (11). Pretreatment with gomisin A was also found to attenuate the activation of caspase-3, elevation of serum TNF-α, the number of apoptotic cells, and DNA fragmentation during D-galactosamine (GalN) and LPS-induced hepatic apoptosis and liver injury (12). The correlation between gomisin A and hepatitis C virus (HCV) infection has been investigated using an *in vitro* MOLT-4 cell model and an *in vivo* animal model of acute hepatic injury. Treatment with gomisin A both short-term and long-term effectively inhibited HCV infection and protected against immunological hepatopathy (13). In a study on liver regeneration, pretreatment with gomisin A stimulated regeneration of liver damaged by partial hepatectomy by increasing ornithine decarboxylase activity, which regulates important biochemical processes in the initial stage of liver regeneration (14). Furthermore, gomisin A was shown to be tightly correlated with hepatocarcinogenesis. Finally, oral administration of gomisin A significantly inhibited the increase in serum bile acids (deoxycholic acid), occurrence of preneoplastic lesions, and the number of GST-P-positive loci in the liver (15-17). Although hepatic and renal disease induced by CCl_4 has been studied extensively, the mechanism by which these organs are protected against damage has not been widely investigated. In addition, there are few studies on whether or not gomisin A protects the liver and kidney against damage induced by CCl_4 exposure.

Therefore, the present study investigated the protective effects of gomisin A against liver and kidney injury induced by CCl_4 exposure. Our results showed that gomisin A significantly inhibited the increase in serum biochemical markers indicative of liver and kidney toxicity, histological damage, and caspase activation through differential regulation of the MAPK signaling pathway.

Materials and methods

Preparation of gomisin A. Fruits of *S. chinensis* used in this study were collected from Moonkyeng City, Korea in September, 2005. A voucher specimen (accession no. SC-PNUNPRL-1) was deposited in the Herbarium of Pusan National University. To purify gomisin A, dried fruits of *S. chinensis* (2.5 kg) were ground into a fine powder and successively extracted at room temperature with *n*-hexane, EtOAc and MeOH. The hexane extract (308 g) was evaporated under vacuum and chromatographed on a silica gel (40 μm; J.T. Baker, Phillipsburg, NJ, USA) column (70x8.0 cm) with a step gradient of 0, 5, 10, 20 and 30% EtOAc in hexane (each 1 liter) (18). Of these extracts, fraction 29 (1,992 mg) was separated on a silica gel column (100x3.0 cm) with 15% $CHCl_3$ in acetone to provide gomisin A (973 mg). Pure gomisin A was identified by HPLC on a Phenomenex Luna C18 column (150x4.6 mm ID; 5 μm particle size; Phenomenex) (19). In addition, the chemical structure of gomisin A used in this study was verified by LC-MS (Bruker BioApex FT mass spectrometer) and NMR analysis (Varian Inova 500 spectrometer) (20) (Fig. 1A).

Care and use of laboratory animals. Sprague-Dawley (SD) rats used in this study were purchased from Samtaco BioKorea (Osan, Korea). All animal experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Pusan National University (approval no. PNU-2009-0007). Animals were handled at the Pusan National University-Laboratory Animal Resources Center accredited by the Korea FDA in accordance with USA NIH guidelines (accredited unit no. 000996). All rats were housed under specific pathogen-free (SPF) conditions with a strict light cycle (lights on at 06:00 h and off at 18:00 h) and were fed a standard irradiated chow diet (Purina Mills, Inc.) *ad libitum*.

Gomisin A treatment and measurement of organ weight. Eight-week-old SD rats were randomly divided into three subgroups with six rats/group. The first group of SD rats was not treated with any compounds (non-treated group). The second group received a comparable volume of olive oil via oral gavage (vehicle/CCl_4-treated group) daily, whereas the third group received 100 mg/kg body weight per day of gomisin A via oral injection for four days (gomisin A/CCl_4-treated group). On the fifth day, the second and third groups received 0.1 ml of CCl_4 solution via intraperitoneal injection. At 24 h after CCl_4 injection, the animals were immediately euthanized using CO_2 gas. Body weights as well as weights of internal organs, including the liver, kidney, heart, lung, spleen and thymus, were measured using a chemical balance. Subsequently, liver and kidney tissues as target organs were collected and stored in Eppendorf tubes at -70˚C until being assayed. For histological analysis, these tissues were fixed in 10% formalin solution for 24 h.

Serum biochemical analysis. After the final administration of CCl_4, all rats were fasted for 24 h, and blood was collected from abdominal veins. Serum was obtained by centrifugation of blood incubated for 30 min at room temperature. Serum biochemical components were assayed using an automatic serum analyzer (Hitachi 747; Hitachi, Japan). All assays were assessed using fresh serum and conducted in duplicate.

Histological analysis. Liver and kidney tissues collected from rats were fixed with 10% formalin for 24 h, embedded in paraffin wax, and then sectioned into 5-μm slices. The liver and kidney sections were then stained with hematoxylin and eosin (H&E; Sigma-Aldrich, St. Louis, MO, USA). The stained liver and kidney tissue sections were observed by light microscopy, and morphological features of hepatocytes and kidney cells were assessed with Leica Application Suite (Leica Microsystems, Switzerland).

Western blot analyses. Proteins prepared from tissues of the vehicle/CCl_4- and gomisin A/CCl_4-treated SD rats were separated by electrophoresis on a 4-20% SDS-PAGE gel for 3 h and then transferred to nitrocellulose membranes for 2 h at 40 V. Each membrane was incubated separately with the primary antibody: anti-caspase-3 (#9662; Cell Signaling Technology,

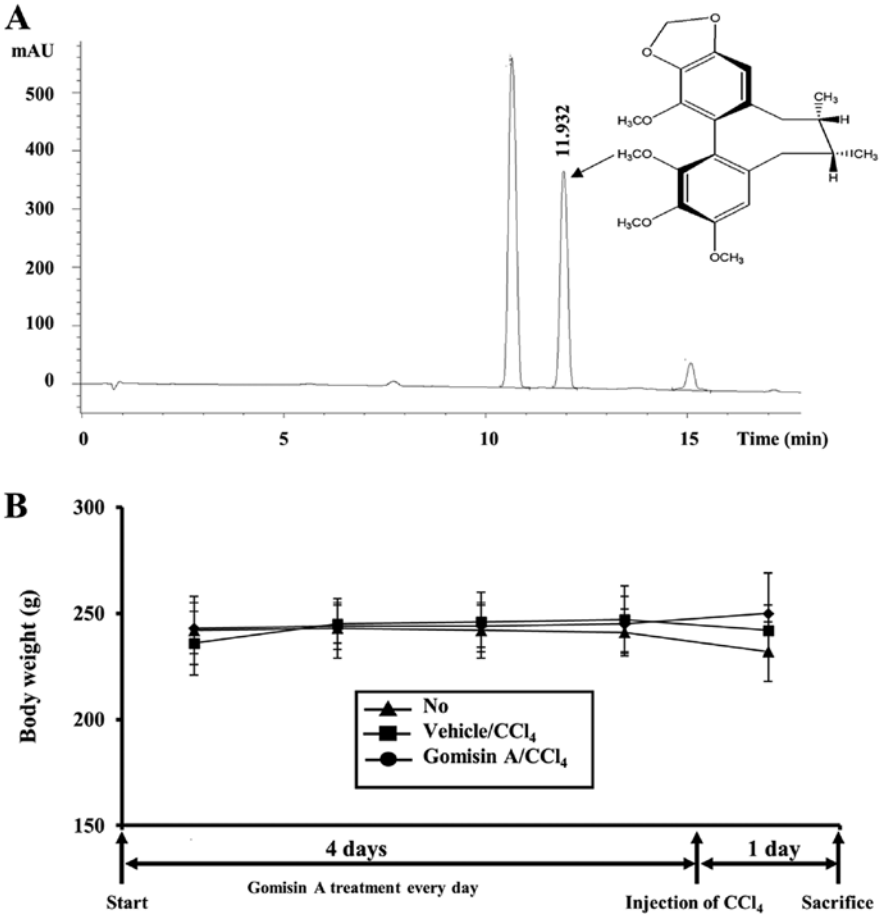

Figure 1. (A) Chromatogram of gomisin A isolated from *Schisandra chinensis*. Purified gomisin A was identified using HPLC and LC-MS as described in Materials and methods. (B) Alteration of body weight during the experimental process. Weight fluctuations throughout 7 days were observed by the daily measurement of body weights. Body weight was measured in triplicate using a chemical balance. Values are the means ± standard deviation.

Boston, MA, USA), anti-Bax (ab7977), anti-Bcl-2 (ab7973; both were from Abcam, Cambridge, UK), anti-ERK (sc-94), anti-p-ERK (sc-7383; both were from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), anti-JNK (#9252), anti-p-JNK (#9251), anti-p38 (#9212), anti-p-p38 (#9211; all were from Cell Signaling Technology), or anti-actin (A5316; Sigma-Aldrich, St. Louis, MO, USA) overnight at 4˚C. The membranes were then washed with washing buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4 and 0.05% Tween-20) and incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG (Zymed Laboratories, Inc., South San Francisco, CA, USA) diluted 1:1,000 at room temperature for 2 h. The membrane blots were developed using a Chemiluminescence Reagent Plus kit (ECL; Pfizer and Pharmacia, New York, NY, USA).

Statistical analysis. Tests for significance between the vehicle- and gomisin A-treated SD rats were performed using a one-way ANOVA test of variance (SPSS for Windows, release 10.10, standard version; SPSS, Chicago, IL, USA). Tests for significance between the non-treated and CCl_4-treated groups (vehicle/CCl_4 and gomisin A/CCl_4) were performed using a post-hoc test (SPSS for Windows, release 10.10, standard version) of variance, and significance levels are provided in the text. All values are reported as the means ± standard deviation. A P-value <0.05 was considered to indicate a statistically significant result.

Results

Protective effects of gomisin A on body and organ weights. Generally, toxic effects on animal and human bodies are confirmed by alterations in body and organ weights (21). In order to investigate the protective effects of gomisin A against CCl_4-induced toxicity, we first measured the body weights of non-treated vehicle- as well as gomisin A-pretreated rats for five days, including one day following CCl_4 exposure. No significant differences in body weight were detected among the three groups (Fig. 1B). However, results of the organ weight analysis were different from those of the body weight analysis. Of the six organs, five organs, including the kidney, heart, lung, spleen and thymus, showed slightly lower weights in the gomisin A/CCl_4-treated group compared to these values in the vehicle/CCl_4-treated group, although these results were not statistically significant. In contrast, the liver weight was significantly higher in the gomisin A/CCl_4-treated group compared to the vehicle/CCl_4-treated group. In addition, the weights of the liver, lung and spleen in the CCl_4-treated group were significantly lower than those in the non-treated group (Fig. 2). Therefore, the present results suggest that

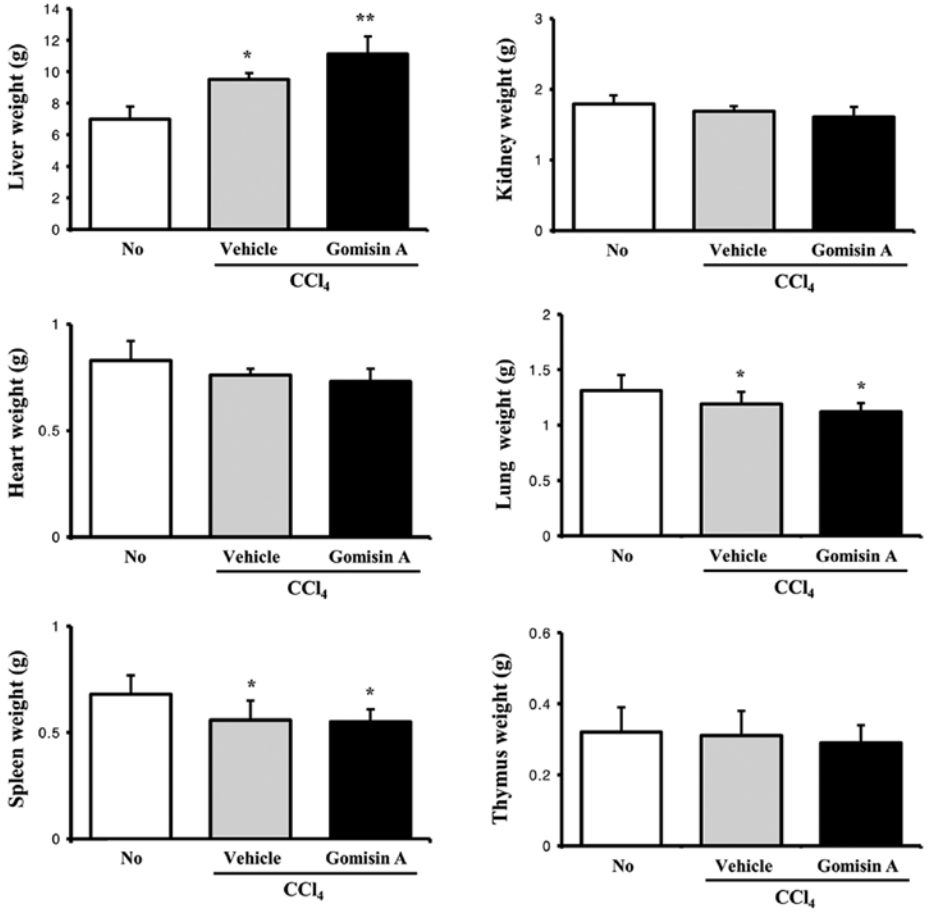

Figure 2. Alteration of organs weights in the vehicle/CCl_4- and gomisin A/CCl_4-treated SD rats. At 24 h after CCl_4 injection, rats were euthanized in a CO_2 chamber, and each organ was collected from the sacrificed SD rats. Their weights were measured in triplicate using a chemical balance. Values are the means ± standard deviation. *P<0.05 is the significance level compared to the non-treated group. **P<0.05 is the significance level compared to the vehicle/CCl_4-treated SD group.

gomisin A did not affect body and organ weights, apart from that of the liver.

Effects of gomisin A on serum biochemical analysis. Increased concentrations of alkaline phosphatase (ALP), alanine transaminase (ALT) and aspartate transaminase (AST) in serum are well-known factors indicating liver toxicity, whereas blood urea nitrogen (BUN) and creatinine (CRE) are evidence of kidney toxicity (21,22). To investigate the protective effects of gomisin A against CCl_4-induced toxicity in terms of serum biochemical indicators, the levels of five indicators, including ALP, AST, ALT, BUN and CRE, were measured in the vehicle/CCl_4- and gomisin A/CCl_4-treated rats. In regards to the liver toxicity factors, the levels of ALP, AST and ALT were higher in the vehicle/CCl_4-treated rats than these levels in the non-treated rats. However, these levels were significantly decreased in the gomisin A/CCl_4-treated rats when compared with the vehicle/CCl_4-treated rats, although the rate of decrease varied for each factor (Fig. 3A). Furthermore, the factors representing kidney toxicity showed similar patterns as those representing liver toxicity. Specifically, serum levels of BUN and CRE were significantly increased upon CCl_4 exposure. However, the concentrations of these two factors were restored by gomisin A pretreatment to similar levels as those in the non-treated group (Fig. 3B). Therefore, these results demonstrated that pretreatment with gomisin A conferred protective effects against liver and kidney damage, although the protective effects varied according to each factor.

Protective effects of gomisin A against tissue injury of the liver and kidney. Generally, histological staining is performed with H&E to visualize the differences between tissue components under normal and pathological conditions. Histological alterations during hepatocellular damage along with the protective effects of gomisin A were first identified by histological analysis of the liver section. In the non-treated group, the histopathology of the liver displayed a normal distribution of hepatocytes with clear visible nuclei, a portal triad and central vein (Fig. 4). However, following CCl_4 treatment, extensive centrolobular necrosis was observed in and around the terminal hepatic venule (THV) of the liver. Furthermore, the central vein was significantly dilated in the vehicle/CCl_4-treated group compared with the non-treated group (Fig. 4). However, the liver section of the group pretreated with gomisin A for four days displayed low hepatocellular necrosis, a poorly dilated central vein, and regular arrangement of hepatocytes.

In the kidney, significant changes were detected only in the cortex containing the Bowman's capsule and convoluted tubules, whereas the medulla region maintained its morphology. Regarding the Bowman's capsule, the vehicle/CCl_4-treated

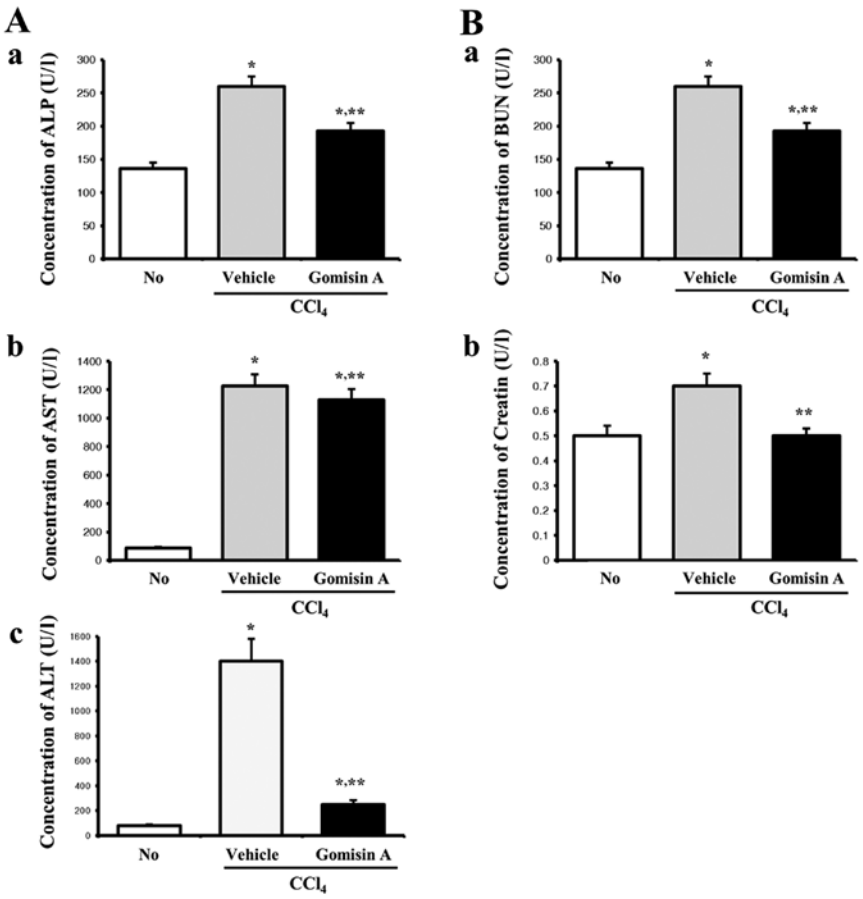

Figure 3. Effects of gomisin A on serum biochemical analysis. Concentrations of (Aa) ALP, (Ab) AST, (Ac) ALT, (Ba) BUN and (Bb) creatinine in serum were analyzed in triplicate using a serum biochemical analyzer as described in Materials and methods. Data represent the means ± standard deviation from three replicates. *P<0.05 is the significance level compared to the non-treated group. **P<0.05 is the significance level compared to the vehicle/CCl_4-treated group.

group showed an increased diameter of the glomerulus as well as a higher number of capillaries in the glomerulus compared with the non-treated group. In the gomisin A pretreatment group, the diameter of the glomerulus, the number of capillaries, and Bowman's space were significantly decreased. In particular, Bowman's space completely disappeared in the gomisin A/CCl_4-treated group (Fig. 4). Regarding the convoluted tubules, their diameters were dramatically increased in the CCl_4-exposed group compared with the non-treated group. However, gomisin A pretreatment induced recovery of the diameters of the convoluted tubules back to normal (Fig. 4). The above results suggest that gomisin A pretreatment contributed to the reduction of hepatic necrosis and dilation of the THV in livers of the SD rats after CCl_4 exposure. Furthermore, this lignan reduced the occurrence of renal defects, including increased diameter of the glomerulus, a higher number of capillaries and convoluted tubules.

Effects of gomisin A on the apoptosis of the liver and kidney. In order to investigate whether or not gomisin A prevents the activation of apoptosis, alterations in apoptosis-related proteins were examined in the liver and kidney tissues of the rats pretreated with vehicle or gomisin A. First, changes in the levels of these proteins were measured in liver tissue. The pro-caspase-3 level was reduced significantly in the vehicle/CCl_4-treated group, whereas the level of active caspase-3 increased. In the gomisin A/CCl_4-treated group, the levels of pro-caspase-3 and active caspase-3 were recovered to the same levels as those of the non-treated group. Bcl-2 belongs to a family of proteins that includes both pro- and anti-apoptotic members. Among these members, Bcl-2 proteins stimulate anti-apoptosis while the Bax protein significantly inhibits the anti-apoptotic actions of the Bcl-2 protein (23,24). To assess the effects of gomisin A pretreatment on proteins associated with the apoptotic signaling pathway, the expression levels of the Bcl-2 and Bax proteins were determined in the vehicle/CCl_4-treated and gomisin A/CCl_4-treated groups using western blot analysis. The expression of the Bax protein was slightly increased in the vehicle/CCl_4-treated group compared to the non-treated group, whereas it was further increased in the gomisin A/CCl_4-treated group. However, the expression level of the Bcl-2 protein was maintained at a certain level regardless of gomisin A pretreatment (Fig. 5). Therefore, western blot analysis indicated that gomisin A reduced the expression levels of proteins associated with anti-apoptosis in the liver tissue.

Additionally, alterations in the expression levels of these proteins were detected in the kidney tissue. The expression levels of pro-caspase-3 and active caspase-3 were markedly increased in the vehicle/CCl_4-treated group, while their levels were decreased in the gomisin A/CCl_4-treated group (Fig. 6). The expression pattern of the Bax protein very much resembled

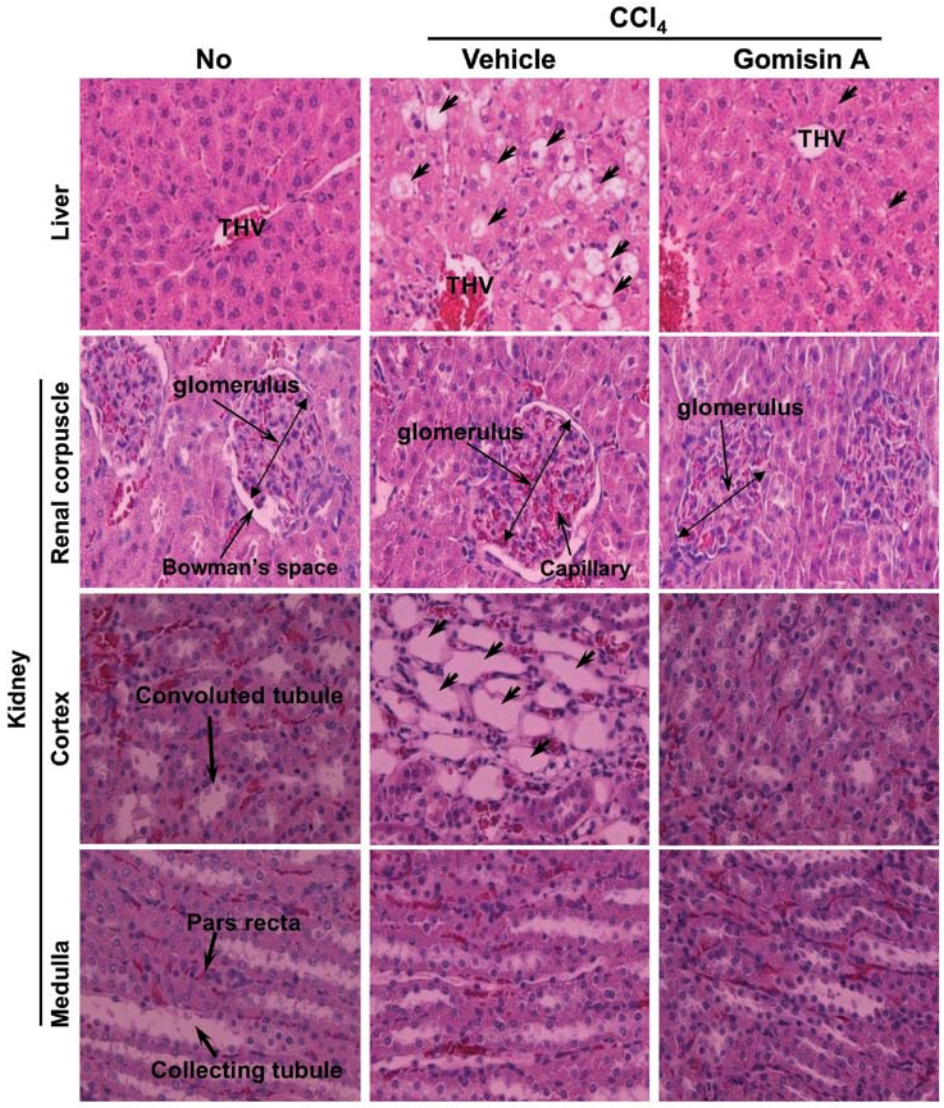

Figure 4. Histology of liver and kidney tissues. Liver and kidney tissues collected from rats were fixed in 4% formalin and stained with H&E solution. Histological phenotypes in each section were observed using Leica Application Suite (Leica Mycrosystems, Switzerland). Liver tissue was mainly observed around the terminal hepatic venule (THV), whereas kidney tissue was observed in three regions: the medulla, cortex, and renal corpuscle; magnification, x200.

its pattern in liver tissue, whereas the pattern of Bcl-2 expression differed from that observed in the liver tissue. Following CCl_4 exposure, the expression of the Bcl-2 protein dramatically increased ~3-fold. However, gomisin A pretreatment prevented an increase in the expression of this protein (Fig. 6). Therefore, these results indicate that gomisin A reduced the expression of proteins associated with anti-apoptosis in the kidney tissue.

Effects of gomisin A on the MAPK signaling pathway. We investigated the roles of different MAPK signaling proteins on CCl_4-induced liver and kidney damage following gomisin A pretreatment. In regards to the liver, the phosphorylation levels of ERK and JNK were decreased in the vehicle/CCl_4-treated group when compared with levels in the non-treated group, whereas the phosphorylation level of p38 did not significantly change. However, in the gomisin A/CCl_4-treated group, the phosphorylation levels of ERK and p38 were significantly higher compared to those in the vehicle/CCl_4-treated group (Fig. 7).

Furthermore, the MAPK signaling pathway in the kidney showed a different response compared to the liver. In the vehicle/CCl_4-treated group, phosphorylation of JNK increased when compared to its level in the non-treated group. In contrast, phosphorylation of p38 was reduced by 50%, whereas the phosphorylation level of ERK was maintained at a constant level. However, in the gomisin A/CCl_4-treatment group, the phosphorylation level of ERK was significantly decreased when compared to that in the vehicle/CCl_4-treated group, whereas phosphorylation of p38 and JNK was increased (Fig. 8). Therefore, these results showed that gomisin A pretreatment had protective effects against liver and kidney damage induced by CCl_4 exposure through differential regulation of the MAPK signaling pathway.

Discussion

The effects of several lignan compounds isolated from *S. chinensis* on chronic liver injury induced by CCl_4 have been

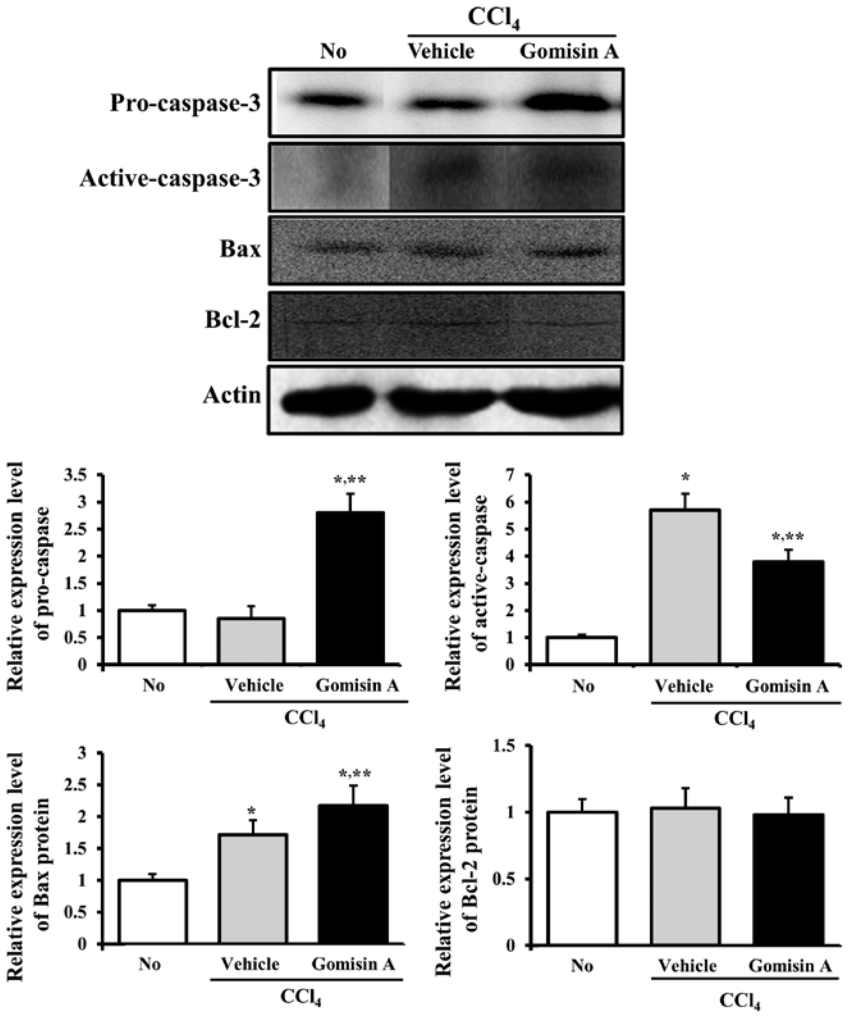

Figure 5. Effects of gomisin A on the apoptotic pathway in the liver. Expression levels of caspase-3, Bcl-2 and Bax proteins in liver tissue were analyzed using western blot analysis. Membranes were incubated with antibodies for caspase-3, Bcl-2 and Bax, as well as β-actin protein from the liver. Expression levels were quantified by an imaging densitometer, and the sizes of the products are indicated, respectively. Data represent the means ± standard deviation from three replicates. *P<0.05 is the significance level compared to the non-treated group; **P<0.05 is the significance level compared to the vehicle/CCl_4-treated group.

previously studied. The first attempt to investigate their effects was performed using gomisin A (TJN-101) (25). Oral administration of gomisin A at a dose of 10 or 30 mg/kg/day for three or six weeks was shown to increase serum biochemical parameters, to suppress fibrosis proliferation, and to accelerate both the repair of liver function and liver regeneration in rats subcutaneously injected with CCl_4. The results observed in this study closely resemble those of the present study, although the treatment time and concentration were quite different. Numerous novel effects of gomisin A on hepatic and renal injury were observed in this study. In particular, the effects of gomisin A on the apoptotic pathway were investigated in terms of the expression levels of caspase-3, Bax and Bcl-2 in the liver and kidney.

Schisandrin B (Sch B), a dibenzocyclooctadiene derivative isolated from *S. chinensis*, was found to exhibit protective effects against CCl_4-induced hepatotoxicity. Enhancement of the hepatic glutathione antioxidant system constituted the first evidence of the protective effects of Sch B against CCl_4-induced toxicity in female Balb/c mice (26). In a comparative experiment, both Sch B and dimethyl diphenyl bicarbonate (DDB) at the same dosage significantly suppressed an increase in plasma ALT activity, although a decrease in plasma SDH activity was observed only in the Sch B-pretreated group (27). In this study, the levels of three indicators, including ALT, AST and ALP, were significantly reduced by gomisin A, as in the Sch B administration study. However, only the therapeutic effects of gomisin A on kidney toxicity were determined in our study. Recently, the molecular mechanism underlying the hepatoprotective effect of Sch B was elucidated. It was shown that Sch B pretreatment induces hepatoprotection against CCl_4-induced liver injury by increasing hepatic mitochondrial resistance to the Ca^{2+}-stimulated permeability transition (28). In our study, gomisin A was shown to function via the apoptotic mechanism and MAPK signaling pathway during chronic liver and kidney injury, although the association between Ca^{2+} permeability and liver protection induced by gomisin A was not investigated.

The effects of several *S. chinensis* extracts on the antioxidant status have been investigated in animals presenting with CCl_4-induced liver injury. A lignan-enriched extract of fruits of *S. chinensis* was shown to facilitate the regeneration of the

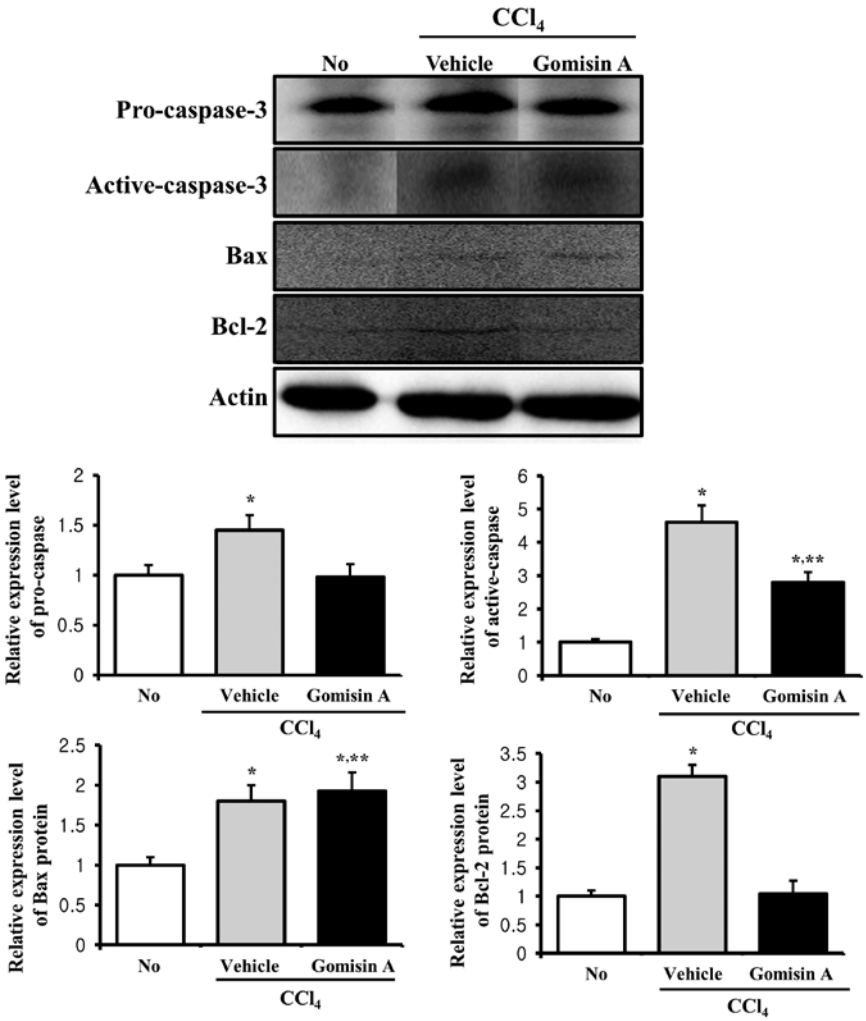

Figure 6. Effects of gomisin A on the apoptotic pathway in the kidney. Expression levels of caspase-3, Bcl-2 and Bax proteins were analyzed using western blot analysis in kidney tissue. Membranes were incubated with antibodies for caspase-3, Bcl-2 and Bax, as well as β-actin protein from the kidney. Expression levels were quantified by an imaging densitometer, and the sizes of the products are indicated, respectively. Data represent the means ± standard deviation from three replicates. *P<0.05 is the significance level compare to the non-treated group; **P<0.05 is the significance level compared to the vehicle/CCl_4-treated group.

hepatic glutathione status through a glutathione reductase-catalyzed and NADPH-mediated reaction (29). In another study, the lignan fraction of *S. chinensis* showed strong protective effects against liver injury induced by CCl_4 during phase I oxidative metabolism (30). Furthermore, the combined herbal extract of *Ginkgo biloba*, *Panax ginseng* and *S. chinensis* was found to significantly improve hepatic antioxidant capacity by increasing catalase activity and the glutathione redox status (31). As shown in the above studies, it is important to investigate the alteration of the antioxidant status in liver tissue in order to verify the protective effects of various lignans. Therefore, more research is needed to further identify the effects of gomisin A.

Reduction in pro-caspase-3 levels results in increased levels of active caspase-3, since the apoptotic signal induces cleavage of pro-caspase-3 (32 kDa) into two small fragments (17 and 12 kDa) (32). In the present study, high levels of active caspase-3 were detected in the vehicle/CCl_4-treated group, and their levels significantly decreased upon gomisin A pretreatment (Figs. 5 and 6). Therefore, these results also confirm that gomisin A may inhibit hepatic and renal apoptosis through suppression of caspase-3 activity.

Apoptosis or programmed cell death plays critical roles in a variety of physiological processes during fetal development as well as in adult life. Defects in the apoptotic process lead to the onset of many diseases involving progressive cell accumulation as well as cancer in most cases. Furthermore, apoptosis involves many families of proteins. Of these, Bcl-2 proteins are one of the key families that induce anti-apoptosis (23). The Bax protein, another member of the Bcl-2 family, inhibits the anti-apoptotic actions of Bcl-2. In order to assess the effects of gomisin A pretreatment on proteins associated with the apoptotic signaling pathway, the expression levels of Bcl-2 and Bax were determined in the vehicle/CCl_4- and gomisin A/CCl_4-treated groups using western blot analysis. The expression of the Bcl-2 protein was markedly decreased only in the kidney of rats pretreated with gomisin A, whereas its level was maintained in the liver (Figs. 5 and 6). However, expression of the Bax protein increased slightly in both organs pretreated with gomisin A. These results indicate that gomisin A simultaneously reduces the expression levels of proteins associated with anti-apoptosis while increasing those of proteins associated with pro-apoptosis.

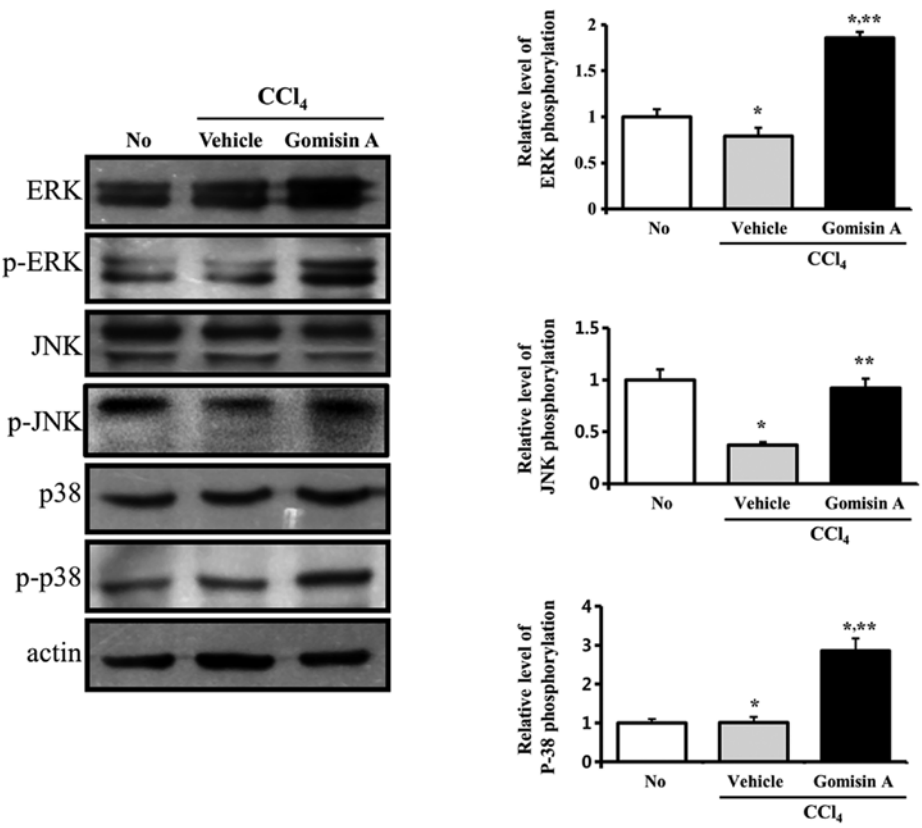

Figure 7. Phosphorylation levels of three MAPK family members in livers of the vehicle- and gomisin A-pretreated rats. To measure the expression levels of each protein, the membranes were incubated with specific antibodies for each protein, as well as β-actin protein from liver lysates. Three rats/group were assayed by western blotting. Values are reported as the mean ± standard deviation. *P<0.05 is the significance level compared to the non-treated group; **P<0.05 is the significance level compared to the vehicle/CCl_4-treated group.

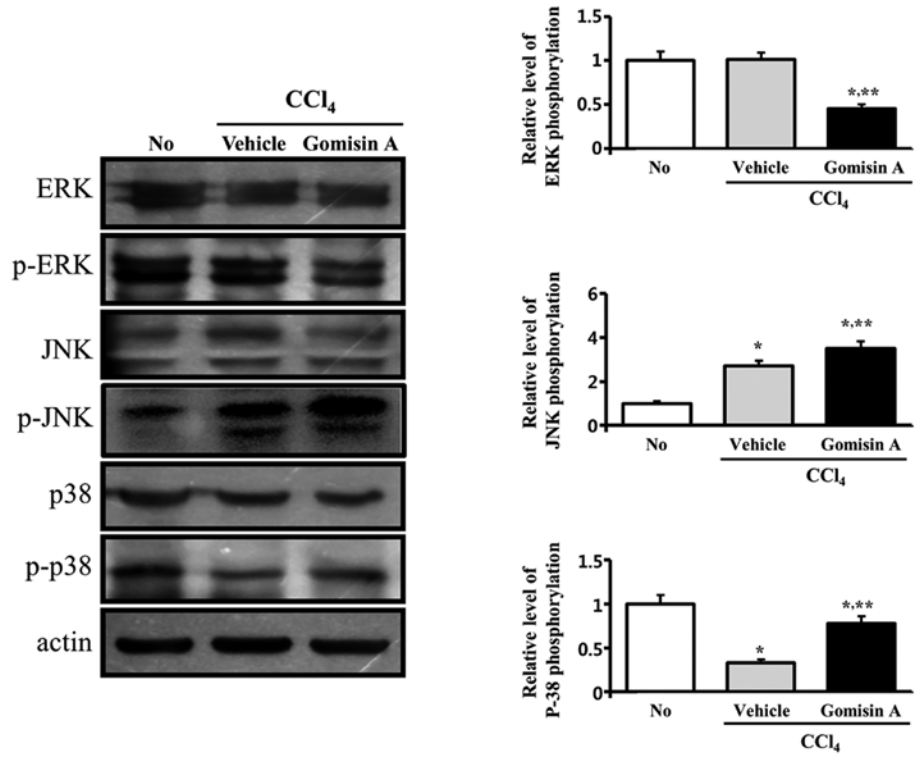

Figure 8. Phosphorylation levels of three MAPK family members in kidneys of the vehicle- and gomisin A-pretreated rats. To measure the expression levels of each protein, the membranes were incubated with specific antibodies for each protein, as well as β-actin protein from kidney lysates. Three rats/group were assayed by western blotting. Values are reported as the means ± standard deviation. *P<0.05 is the significance level compared to the non-treated group; **P<0.05 is the significance level compared to the vehicle/CCl_4-treated group.

The MAPK family is involved in the control of growth and differentiation, as well as in apoptotic signaling (33-35). Members of the MAPK pathway, including ERK1/2, JNKs and p38 MAP kinase (p38), have been well characterized in various studies. In particular, several studies have shown that MAPK signaling proteins are activated by different stimuli. For instance, p38 and JNK are activated in response to many cytotoxic stresses such as hydrogen peroxide (H_2O_2), UV radiation, tumor necrosis factor (TNF-α), heat shock and X-rays (36-38). Furthermore, ERK is activated by various growth factors and mitogens during the processes of cell differentiation, growth and survival (36). The results of this study were significantly different from those of the previous one (36). CCl_4 as a cytotoxic stressor induced an increase in JNK phosphorylation only in the kidney, whereas its level was decreased in the liver (Figs. 7 and 8). However, significant decreases in the phosphorylation level of ERK were detected in the liver and kidney tissues of the vehicle/CCl_4-treated rats. These results suggest that the mechanism of action of CCl_4 differs from that of other cytotoxic stressors affecting the liver and kidney. In particular, pretreatment with gomisin A dramatically increased the phosphorylation of ERK and p38 in the liver, whereas increased JNK and p38 phosphorylation was observed in the kidney.

Taken together, our results revealed that gomisin A is a potential therapeutic compound for the protection and regeneration of the liver and kidney upon injury induced by CCl_4.

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