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Gentiana manshurica Kitagawa Reverses Acute Alcohol-Induced Liver Steatosis through Blocking Sterol Regulatory Element-Binding Protein-1 Maturation

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This study was undertaken to investigate the protective effects of *Gentiana manshurica* Kitagawa (GM) on acute alcohol-induced fatty liver. Mice were treated with ethanol (5 g/kg of body weight) by gavage every 12 h for a total of three doses to induce acute fatty liver. Methanol extract of GM (50, 100, or 200 mg/kg) or silymarin (100 mg/kg) was gavaged simultaneously with ethanol for three doses. GM administration significantly reduced the increases in serum ALT and AST levels, the serum and hepatic triglyceride levels, at 4 h after the last ethanol administration. GM was also found to prevent ethanol-induced hepatic steatosis and necrosis, as indicated by liver histopathological studies. Additionally, GM suppressed the elevation of malondialdehyde (MDA) levels, restored the glutathione (GSH) levels, and enhanced the superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) activities. The concurrent administration of GM efficaciously abrogated cytochrome P450 2E1 (CYP2E1) induction. Moreover, GM significantly reduced the nuclear translocation of sterol regulatory element-binding protein-1 (nSREBP-1) in ethanol-treated mice. These data indicated that GM possessed the ability to prevent ethanol-induced acute liver steatosis, possibly through blocking CYP2E1-mediated free radical scavenging effects and SREBP-1-regulated fatty acid synthesis. Especially, GM may be developed as a potential therapeutic candidate for ethanol-induced oxidative damage in liver.

KEYWORDS: ***Gentiana manshurica*** **Kitagawa; alcoholic steatosis; oxidative stress; CYP2E1; SREBP1**

INTRODUCTION

Alcohol has been the most commonly abused recreational agent in the world since the beginning of civilization, and alcoholic liver disease is a major cause of death from liver diseases all over the world. Over the past 20 years, there has been evidence of a striking increase in alcohol consumption and related problems in China, with the potential for a major impact on world health (*1*). Although great progress has been made in eliminating the pathogenesis of alcoholic liver disease, current therapies for this disease are not effective. Thus, developing novel therapeutic agents such as correcting the fundamental cellular disturbances from excessive alcohol consumption is extremely urgent.

Excessive alcohol consumption usually causes various hepatic dysfunctions, ranging from fatty liver or liver inflammation to focal necrosis and fatty liver terminal venular sclerosis, that can ultimately develop into cirrhosis and even liver cancer (*2*, *3*). Steatosis refers to the fat accumulation in hepatocytes, accompanying the fatty infiltration, the first manifestation of ethanol-induced liver injury. The underlying cause of fat accumulation in alcoholic fatty liver disease is mostly due to the excessive synthesis of fatty acids, inhibition of fatty acid oxidation, and interference with the triglyceride cycle (*4*). Sterol regulatory element-binding proteins (SREBP) belong to a family of membrane-bound transcription factors that control the synthesis and uptake of cholesterol, fatty acids, triglycerides, and phospholipids (*5*). Upon sterol deprivation, SREBP processes a sequential two-step proteolytic cleavage to release the amino-terminal portion SREBP into the nucleus to activate the transcription of genes involved in cholesterol and fatty acid synthesis by binding to sterol regulatory elements (*6*−*8*). Recently, Yin et al. (*9*) reported that acute ethanol administration affects the expression levels of SREBP-1 and many other SREBP target genes, thereby increasing fatty acid synthesis. Therefore, activation of SREBP-1 by ethanol feeding is associated with increased expression of hepatic lipogenic genes such as the accumulation of triglyceride and reduced fatty acid oxidation in the development of an alcohol-induced fatty liver (*10*). Moreover, ethanol is metabolized to acetaldehyde in the liver by alcohol dehydrogenase; acetaldehyde is further oxidized to acetate by aldehyde dehydrogenase and then changed into carbon dioxide via the citric acid cycle. Many processes and factors are thought to be involved in alcoholic liver disease, including oxidative stress. These pathways result in reactive oxygen species (ROS) generation and cause oxidative stress, in which the microsomal ethanol-oxidizing system, especially cytochrome P4502E1 (CYP2E1), plays a critical role in ethanol-induced oxidative stress and liver damage. There is increasing evidence that oxidative stress plays an important etiologic role in the pathogenesis of ethanol-induced

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liver injury. Induction of CYP2E1 by ethanol is a key pathway, and, importantly, pathological changes in ethanol-induced liver injury correlate with CYP2E1 levels (*11*). It was reported that ethanol-induced liver injury was related with enhanced lipid peroxidation, formation of lipid radicals, and decreased hepatic antioxidant defense, especially glutathione (*12*).

Traditional herbs have attracted world attention as health-beneficial foods and become source materials for drug development. Herbal medicines isolated from plants or fruits have been used to treat the alcoholic fatty liver, and their protective effects were believed to be associated with the antioxidant activity (*13*, *14*) and the inhibition of SREBP-1c maturation (*15*, *16*).

Gentiana manshurica Kitagawa (GM) is abundantly distributed in northeastern China and known as "Dongbeilongdan" and has been used traditionally as a folk remedy by Chinese people suffering from chronic liver disease. As an iridoid-containing plant, GM has various pharmacological activities. Previous phytochemical studies reported that GM includes loganic acid, 6-*O*-β-D-glucopyranosylgentiopicroside, swertiamarin, gentiopicroside, sweroside, and 2-(*o*,*m*-dihydroxybenzyl) sweroside (*17*). Among them, gentiopicroside is a major bioactive herbal ingredient isolated from GM. Previously, we have reported that gentiopicroside-containing GM extract has a hepatoprotective effect on acetaminophen-induced liver injury (*18*), and gentiopicroside also can protect D-galactosamine/lipopolysaccharide-induced fulminant hepatic failure (*19*). Therefore, we were intrigued as to whether GM could reverse acute alcoholic fatty liver. The current experiment was designed to investigate the protective effects of GM on acute ethanol-induced fatty liver. Here we selected silymarin as a positive control because it was reported that silymarin supplementation significantly attenuated acute ethanol administration (*20*). In this study, we aimed to investigate the antioxidant activity and antifatty liver effects of GM against ethanol exposure compared with silymarin.

MATERIALS AND METHODS

Chemical. Silymarin, which was used as reference drug for in vivo experiments, was purchased from Aldrich Chemical Co. Inc. (Milwaukee, WI). Silymarin was a mixture of seven isomers as follows: toxifolin (4.2%), silybin B (34.3%), silychristin (27.2%), isosilybin A (6.6%), silydianin (3.2%), isosilybin B (1.2%), and silybin A (22.6%). Detection kits for glutathione (GSH), malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT) assay kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). All other chemicals used were of analytical grade.

Preaparation of *G. manshurica* Kitagawa. The GM used in this study was a methanol extract obtained from the rhizomes and roots of *G. manshurica* Kitagawa (GM) and was purchased from Yanbian Puhe, China, in March 2006. Briefly, the rhizomes and roots of GM (1 kg) were extracted three times with methanol (10 L) and boiled under reflux for 4 h at 40 °C, and then the percolate was concentrated in a rotary vacuum evaporator followed by lyophilization. The freeze-dried extract was analyzed on HPLC to confirm the presence of gentiopicroside. In a previous study we determined quantitatively the content of gentiopicroside in methanolic extract by HPLC analysis (2.48%) (*18*). The extract was presolubilized in distilled saline for the in vivo studies.

Animals and Treatment. Male C57BL/6 mice were obtained from the Animal Division of Jilin University (Jilin, China). The mice were housed in the animal quarters at the College of Pharmacy of Yanbian University. They were maintained at 22 °C on a 12:12 h light−dark cycle, and they had free access to rodent chow and tap water. The experimental procedures were approved by the Institutional Animal Care and Use Committee, which is certified by the American Association of Accreditation of Laboratory Animal Care. The mice were divided into six groups randomly, including one control group, one ethanol group, three GM groups (50, 100, and 200 mg/kg of body weight), and one silymarin groups (100 mg/kg of body weight). Mice were treated intragastrically with ethanol (5 g/kg of body weight) every 12 h for a total of three doses. Control mice received an isocalorical maltose solution. In the GM or silymarin group, GM or silymarin was gavaged simultaneously with ethanol. At 4 h after the last dosing, the mice were anesthetized and blood samples were taken for serum biochemistry. The liver was dissected, weighed, frozen in liquid nitrogen, and then stored at −80 °C until analyzed.

Blood Biochemistry. Blood was collected at 4 h after the last ethanol administration. Serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were detected by using an Autodry Chemistry Analyzer (SPOTCHEM SP4410, Arkray, Japan).

Determination of the Serum and Hepatic Triglyceride Levels. Serum triglyceride (TG) levels were detected by using an Autodry Chemistry Analyzer (SPOTCHEM SP4410, Arkray). For determination of hepatic TG content, 100 mg of liver was homogenized in 4 mL of chloroform/methanol (2:1, v/v). A total of 0.8 mL of 50 mM NaCl was added to each sample. Samples were then centrifuged, and the organic layer was removed and dried. The resulting pellet was dissolved in phosphate-buffered saline containing 1% Triton X-100, and the triglyceride contents were determined using a commercially available enzymatic reagent kit (Sigma Chemical Co.). The serum and hepatic TG concentration was expressed as millimolar and milligrams per gram of liver, respectively.

Hepatic Lipid Peroxidation Assay and Antioxidant System Examination. Liver tissue was homogenized in 9 volumes of cold buffer (0.01 mM Tris-HCl, 0.1 mM EDTA, 0.01 M saccharose, and 0.8% saline, pH 7.4) at 4 °C. Then the homogenates were centrifuged at 4 °C (3000 rpm/min, 15 min), and the supernatant was stored for determination. Protein content was determined using a BCA protein assay kit (Beyotime, Jiangsu, China). The levels of GSH and MDA and activities of SOD, GPX, and CAT were measured using commercial assay kits (Nanjing Jiancheng Institute, China) according to the manufacturer's instructions.

Histopathological Analysis. Liver samples obtained at 4 h after the last ethanol administration were fixed in 10% phosphate-buffered formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin for histopathological analysis. The sections were examined by light microscopy.

Preparation of Total Protein and Isolation of Nuclear Fractions from Liver. The total protein extracts were made by pulverization in a grinder with liquid nitrogen and then using a ratio of 1 mL of lysis buffer (150 mM NaCl, 1.0% NP-40, 0.5% $NaVO_4$, 0.1% SDS, 50 mM Tris, pH 7.5) containing 1 mM PMSF for each 100 mg of powdered liver sample. The isolation of nuclear fractions from mice liver pulverized into a powder while frozen was performed using the NE-PER Nuclear and Cytoplasmic Extraction kit (Pierce Biotechnology, Rockford, IL) according to the manufacturer's instructions.

Immunoblot Analysis. The protein from nuclear or total protein was separated by 10% sodium dodecyl sulfate−polyacrylamide gel electrophoresis and transferred to PVDF membranes by electroblotting. The membranes were blocked in 5% skim milk in PBST (0.05% Tween 20 in phosphate-buffered saline). The blots were incubated overnight with CYP2E1 and SREBP-1 antibodies diluted in 5% skim milk in PBST at 4 °C followed by incubation with secondary antibodies conjugated to horseradish peroxidase (diluted in 5% skim milk powder in PBST) for 1 h at room temperature. Proteins were detected by enhanced chemiluminescence using WEST-ZOL(plus) Western blot detection system (iNtRON Biotechnology Co., Seongnam, Korea), and band densitometry was performed with Quantity One software (Bio-Rad, USA).

Statistical Analysis. Values reflect means ± SD. One-way ANOVA and Tukey's multiple-comparison tests were performed with the GraphPad Prism program (Graphpad Software, Inc., San Diego, CA). p values of < 0.05 were considered to be statistically significant.

RESULTS

Effect of GM on Ethanol-Induced Hepatotoxicity. The effects of pretreatment with GM on the ethanol-induced elevation of serum AST and ALT activities are shown in panels **A** and **B**, respectively, of **Figure 1**. Three doses of ethanol (5 g/kg) caused hepatotoxicity in mice, as indicated by the increases in serum ALT and AST levels after ethanol administration. GM treatment prevented the ethanol-induced elevation of serum ALT and AST levels in a dose-dependent manner (**Figure 1A**,**B**). Liver histology was normal except for the presence of faint microvesicular steatosis

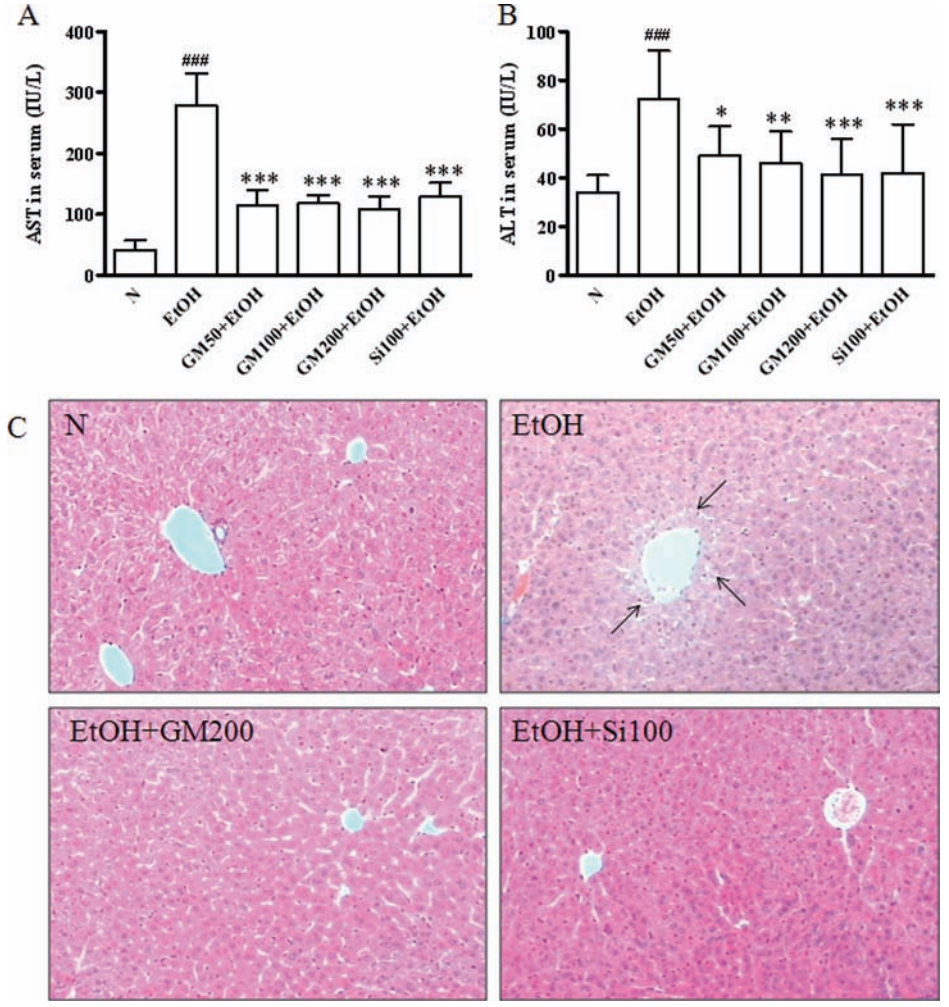

Figure 1. Effect of GM on ethanol-induced hepatotoxicity. Mice were treated with ethanol (5 g/kg) by gavage every 12 h for a total of three doses to induce acute fatty liver. GM (50, 100, or 200 mg/kg) or silymarin (100 mg/kg) was gavaged simultaneously with ethanol for three doses. Serum AST (**A**) and ALT (**B**) and histopathological analyses (**C**) were performed at 4 h after the last ethanol administration, and each value is expressed as the mean ± SD ($n = 10$). Black arrows indicate fat droplets in liver sections in the liver. ###, $p < 0.001$, significantly different when compared with control group; ∗, $p < 0.05$, ∗∗, $p < 0.01$, and ∗∗∗, $p < 0.001$, significantly different when compared with ethanol alone group.

in the ethanol-treated group. Acute ethanol exposure caused degenerative morphological changes exhibited by fat droplets in liver sections in the liver. These alcohol-induced hepatic pathological changes were significantly inhibited in GM-pretreated mice (**Figure 1C**).

Effects of GM on Serum and Hepatic Triglyceride Levels. Ethanol administration induced significant accumulation of TG in the liver. GM pretreatment obviously inhibited the increase of the serum and hepatic TG levels, and the effects of GM are comparable to those of silymarin (**Figure 2**). These results indicate that GM might be effective against alcoholic steatosis.

Effects of GM on the Content of MDA, GSH Levels, and Activities of GPX, SOD, and CAT. To evaluate the effect of GM pretreatment on ethanol-induced liver lipid peroxidation, we monitored the levels of MDA, an indicator of oxidative damage and one of the principal products of lipid peroxidation. As shown in **Figure 3**, MDA production in the ethanol-treated group significantly increased 2.7-fold compared to the control ($p < 0.001$). Consistent with the serum levels of ALT and AST, GM pretreatment significantly decreased the ethanol-induced hepatic lipid peroxidation in a dose-dependent manner (**Figure 3**). Briefly, in mice receiving GM (50, 100, or 200 mg/kg) plus ethanol, the MDA levels were significantly reduced to 50% ($p < 0.001$), 47% ($p < 0.001$), or 43% ($p < 0.001$) of those of solely ethanol-treated mice. Comparable levels of MDA were measured in mice that received silymarin (100 mg/kg) or GM (50, 100, or 200 mg/kg). Ethanol-treated mice exhibited significant decreases in hepatic GSH concentrations to 60% compared to control mice ($p < 0.05$). Pretreatment with a high dose of GM (200 mg/kg) significantly inhibited the GSH depletion produced by ethanol ($p < 0.01$) (**Figure 3**). Meanwhile, 4 h after ethanol administration, the ethanol group had significantly decreased GPX, SOD, and CAT activities to 39% ($p < 0.001$), 58% ($p < 0.001$), and 56% ($p < 0.001$) compared to the control, respectively. However, GPX ($p < 0.01$), SOD ($p < 0.001$), and CAT ($p < 0.001$) activities were significantly enhanced in the group treated with 200 mg/kg GM plus ethanol (**Figure 3**).

Effects of GM on CYP2E1 Expression. In mice, GM pretreatment resulted in a dose-dependent protective effect against ethanol-induced hepatotoxicity. By immunoblot analysis, we examined the effects of GM on CYP2E1 protein expression. As shown in **Figure 4**, ethanol administration increased the CYP2E1 content by 2.5-fold compared to control at 4 h after the final ethanol dosing, but the elevation of CYP2E1 expression in alcoholic liver was inhibited significantly by GM. The results suggest that the suppression of CYP2E1 by GM in mice is an important aspect of the hepatoprotective effect of GM against ethanol.

Effects of GM on Maturation of Nuclear SREBP-1 Involved in Fatty Acid Synthesis. On the basis of the evidence that SREBP-1 is

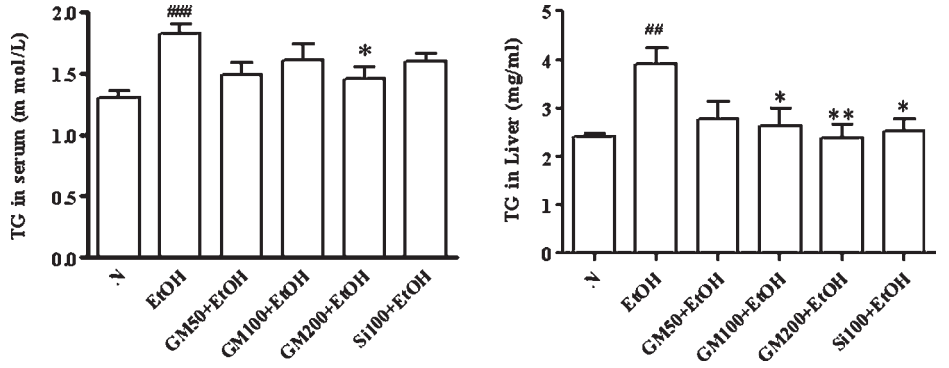

Figure 2. Effect of GM on serum and hepatic TG levels. Mice were treated with ethanol (5 g/kg, body weight) by gavage every 12 h for a total of three doses to induce acute fatty liver. GM (50, 100, or 200 mg/kg) or silymarin (100 mg/kg) was gavaged simultaneously with ethanol for three doses. Serum and hepatic TG contents were measured at 4 h after the last ethanol administration. ##,$p < 0.01$, and ###, $p < 0.001$, significantly different when compared with control group; ∗, $p < 0.05$, and ∗∗,$p < 0.01$, significantly different when compared with ethanol alone group.

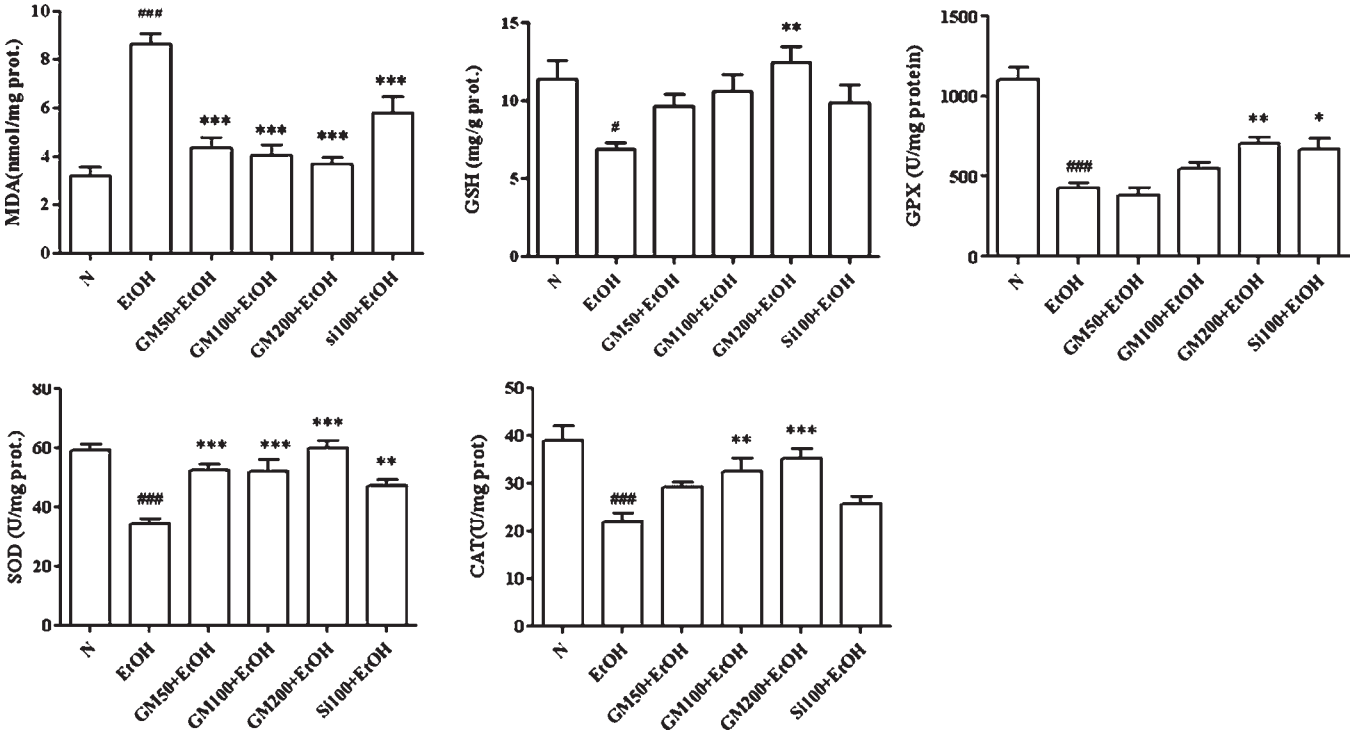

Figure 3. Effects of GM on the hepatic antioxidant system. Mice were treated with ethanol (5 g/kg of body weight) by gavage every 12 h for a total of three doses to induce acute fatty liver. GM (50, 100, or 200 mg/kg) or silymarin (100 mg/kg) was gavaged simultaneously with ethanol for three doses. #, $p < 0.05$, and ###, $p < 0.001$, significantly different when compared with control group; ∗, $p < 0.05$, ∗∗, $p < 0.01$, and ∗∗∗, $p < 0.001$, significantly different when compared with ethanol alone group.

involved in hepatic lipid homeostasis, we tested the effects of GM treatment on the maturation and nuclear translocation of SREBP-1 in ethanol-induced liver injury. As shown in **Figure 5**, ethanol-induced nuclear translocation of mature SREBP-1 (nSREBP-1) was also observed in the ethanol group. Treatment of both ethanol and GM significantly inhibited nSREBP-1 maturation and its translocation to the nucleus. In particular, SREBP-1c mediates insulin effects on lipogenic gene expression in both adipocytes and liver (*21*, *22*). These results demonstrated that the inhibition of the maturation of nSREBP-1c may contribute to the antisteatosis effects of GM in ethanol-fed mice.

DISCUSSION

Ethanol-induced liver damage progresses through alcoholic fatty liver, alcoholic hepatitis, fibrosis, and cirrhosis. The animal model for acute ethanol-induced hepatotoxicity in human drinkers was previously developed (*23*), and we applied it with some modification according to that given in Song et al. (*20*). In the current experiments, C57BL/6 mice were orally administered three doses of 5 g/kg ethanol. Accumulation of fat is the earliest and most common response to heavy alcohol intake. Alcoholic fatty liver is usually characterized by the elevation of serum transaminase and hepatic and serum TG levels, together with a lot of fat droplets in the liver sections. Our results showed that acute ethanol administration in the mice caused prominent microvesicular steatosis along with necrosis in the liver, which corresponded to the elevation of serum ALT and AST activities and the increase of serum and hepatic TG levels, suggesting that acute ethanol administration induced typical fatty liver (**Figures 1** and **2**). However, GM effectively suppressed these adverse effects. Parallel

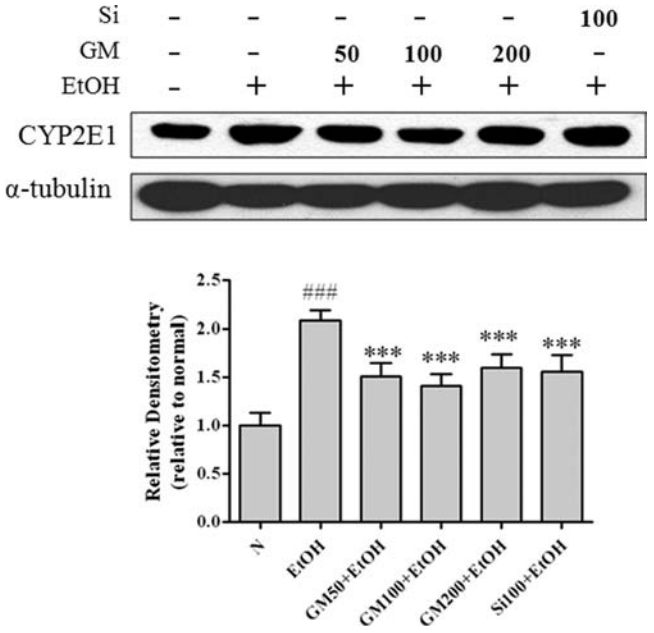

Figure 4. Effects of GM on CYP2E1 protein expression. Mice were treated with ethanol (5 g/kg of body weight) by gavage every 12 h for a total of three doses and simultaneously gavaged with or without GM (50, 100, or 200 mg/kg) or silymarin (100 mg/kg). The liver CYP2E1 protein levels were determined at 4 h after the last ethanol administration by Western blotting. Each immunoreactive band was digitized and expressed as a ratio of α-tubulin levels. The ratio of the normal group band was set to 1.00. Values of densitometric analysis were mean ± SD of three independent experiments. ###, $p < 0.001$, significantly different when compared with control group; ***, $p < 0.001$, significantly different when compared with ethanol alone group.

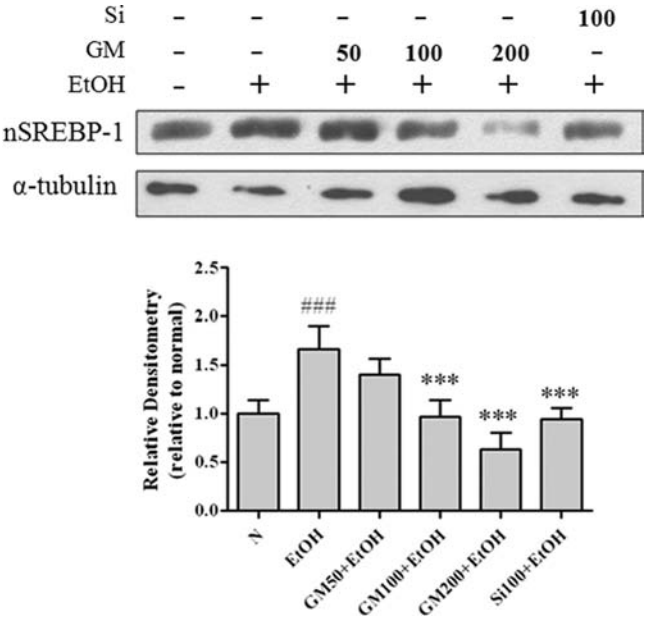

Figure 5. Effects of GM on nuclear translocation of mature SREBP-1. Mice were treated with ethanol (5 g/kg of body weight) by gavage every 12 h for a total of three doses and simultaneously gavaged with or without GM (50, 100, or 200 mg/kg) or silymarin (100 mg/kg). The SREBP-1 protein in nuclear fraction was determined at 4 h after the last ethanol administration by Western blotting. Each immunoreactive band was digitized and expressed as a ratio of α-tubulin levels. The ratio of the normal group band was set to 1.00. Values of densitometric analysis were mean ± SD of three independent experiments. ###, $p < 0.001$, significantly different when compared with control group; ***, $p < 0.001$, significantly different when compared with ethanol alone group.

with these changes, histological examination showed few droplets in GM-treated mice liver (**Figure 1C**). These data strongly indicated that the mice simultaneously treated with GM and ethanol can effectively prevent fatty liver induced by acute ethanol exposure.

It has been documented that alcohol administration causes intracellular accumulation of ROS; thus, oxidative stress plays an important role in acute and chronic alcoholic liver injury, especially when the liver has less antioxidant protection to cope with the ROS generation (*24*, *25*). Oxidative stress is generally considered the result of the disequilibrium between pro-oxidants and antioxidants in biological systems (*26*). Once this imbalance appears, lipid peroxidation occurs. Lipid peroxidation caused by free radicals is considered to be one of the most important factors in the progression of ethanol-induced liver injury. MDA is one of the major end-products of oxidation of polyunsaturated fatty acids and has been frequently used as an indicator of lipid peroxidation and oxidative stress in vivo (*27*). A marked increase of MDA was noted after acute ethanol administration in our study, and this increase was significantly attenuated by GM supplementation, indicating that the antifatty liver effects of GM may be associated with antioxidant activities.

GSH is the most abundant low molecular weight substrate for glutathione-related enzymes. GSH plays an important role in the antioxidant defense system (*28*). Videla et al. (*29*) reported that acute and chronic ethanol ingestion decreases GSH levels and increases lipoperoxidation in the liver both in experimental animals and in humans. GSH deficiency results in cellular damage associated with severe mitochondrial degeneration in the liver. It has been indicated that GSH restoration could inhibit oxidative damage and improve alcohol-induced liver injury (*30*−*32*). The decrease of GSH reduces the antioxidative capacity and increases the sensitivity to oxidative stress. GSH effectively scavenges free radicals and oxidizes them into oxidized glutathione (GSSG), which can be reduced to GSH by GSH reductase with the consumption of NADPH (*33*). Besides this, GPX catalyzes the reduction of hydrogen peroxide and other peroxides. Besides the GSH-related antioxidant system, SOD is also accepted as an important factor in protection against oxidative damage. SOD can catalyze the clearance of the superoxide anion radicals and prevent the formation of H_2O_2 (*34*). The results from our present study indicated that the GM treatment could effectively alleviate alcohol-induced GSH depletion in the liver, which consequently enhanced the activity of GPX, SOD, and CAT (**Figure 3**). The results from our present study coincide with those of previous studies and indicate that oxidative damage was an important cause, and highly responsible for, acute alcoholic liver injury. The preventive effects of GM against alcoholic hepatotoxicity could be partially related with the elevated antioxidant capacity.

CYP2E1 plays a critical role in the ethanol generation of oxidative stress and metabolizes many other toxicologic compounds (*35*−*37*). Among many potential sources of ROS in response to acute ethanol exposure, CYP2E1 contributed to ROS production during its catalytic cycle, and its levels are elevated by ethanol administration (*38*). Therefore, ethanol administration elevates CYP2E1 activity, which facilitates the catalytic reaction from ethanol to acetaldehyde and causes ROS overproduction (*39*, *40*). Thus, in regard to the importance of CYP2E1 for the bioactivation of toxicants, any agent with CYP2E1 inhibitory ability may block upstream ethanol metabolism and reverse ethanol-induced hepatotoxicity and, possibly, fat accumulation. As observed in our present study, GM supplementation depressed

subsequent ethanol-induced elevation of CYP2E1, which may have a role in the inhibition of lipid peroxidation and liver injury (**Figure 4**). These results suggested that the protective action of GM is partially due to its suppressive effect on CYP2E1 activity.

SREBP is a membrane-bound transcription factor that regulates lipid homeostasis by controlling the expression of genes for fatty acid metabolism, as biomarkers for acute ethanol-induced steatosis (*41*). Three members of the SREBP family are produced by two genes, designated SREBP-1 (isoforms 1a and 1c) and SREBP-2 (*42*). In the liver, SREBP-1c is the predominately expressed form of SREBP-1 (*5*) and regulates the synthesis of fatty acids (*43*). As can be seen in **Figure 5**, the increase in serum and hepatic triglyceride level agrees well with the expression of nuclear SREBP-1. Recently, You et al. (*10*) have reported that the metabolism of ethanol increased hepatic lipogenesis by activating SREBP-1 and that this effect of ethanol may contribute to the development of an alcoholic fatty liver. Yin et al. (*9*) first reported that the expression of SREBP-1 is increased in an acute ethanol-induced hepatic steatosis animal model, thereby increasing fatty acid synthesis. As anticipated, ethanol-induced maturation of nSREBP-1 was inhibited by GM. The regulation of SREBP-1 is of special interest because it is the rate-limiting enzyme involved in fatty acid synthesis.

In summary, GM dramatically prevented ethanol-induced acute hepatosteatosis against simultaneous ethanol intake, and its protective effects were comparable to those of silymarin. The preventive effects, at least partly, may be attributed to antioxidant activities and control of the progression of simple steatosis.

ABBREVIATIONS USED

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAT, catalase; CYP2E1, cytochrome P4502E1; GPX, glutathione peroxidase; GSSG, oxidized glutathione; GSH, glutathione; MDA, malondialdehyde; ROS, reactive oxygen species; SREBP, sterol regulatory element-binding proteins; SOD, superoxide dismutase; TG, triglyceride.

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