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Original Article

Hepatoprotective effects of *Solanum nigrum* against ethanol-induced injury in primary hepatocytes and mice with analysis of glutathione S-transferase A1

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Abstract

Background: *Solanum nigrum* is a herbaceous perennial plant, which is widely used in traditional medicine systems for its antioxidant, anti-ulcerogenic, antitumorigenic, and anti-inflammatory characteristics. The purpose of this study was to investigate the protective effects of *S. nigrum* against alcoholic liver damage in primary hepatocytes and mice, using glutathione S-transferase alpha 1 (GSTA1) as an indicator.
Methods: Primary hepatocytes were obtained by the inverse perfusion method improved on Seglen two-step perfusion *in situ*.
Results: In the presence of *S. nigrum* aqueous extracts (100 μg/mL), no hepatocytic damage was observed in cells treated with ethanol, compared with the model group, and GSTA1 ($p < 0.01$) was more sensitive than alanine aminotransferase and aspartate aminotransferase ($p < 0.05$). Mice that received *S. nigrum* aqueous extracts (150 mg/kg) with ethanol showed marked attenuation of ethanol-induced hepatotoxicity, as evidenced by significant reductions of serum transaminases ($p < 0.01$), and variation of hepatic oxidative indices ($p < 0.05$) and GSTA1 ($p < 0.05$), compared with the model group and mice that received *S. nigrum* aqueous extracts (200 mg/kg). All the detection indexes were significantly different ($p < 0.01$) from those of the model group, and the protective effects were almost the same as that of the positive drug group.
Conclusion: These results suggested that *S. nigrum* has hepatoprotective effects against ethanol-induced injury both *in vitro* and *in vivo*, and can protect the integrity of hepatocytes and thus reduce the release of liver GSTA1, which contributes to improved liver detoxification.

Keywords: ethanol; glutathione S-transferase alpha 1; hepatic injury; primary hepatocytes; *Solanum nigrum*

1. Introduction

Solanum nigrum (SN) is a species in the family Solanaceae, native to Eurasia and introduced in America and Australia.[1] It is an herbaceous perennial plant, which is widely used in traditional medicine systems for its antioxidant, anti-ulcerogenic, antitumorigenic, and anti-inflammatory characteristics.[2] The plant has two significant alkaloids, solamargine and solasonine, that produce carbohydrate glycone solasodine, which is bioactive and in great demand in the pharmaceutical industry.

Alcoholic liver damage is one of the most common hepatic injuries worldwide. However, ethanol abuse and dependence are becoming increasingly more serious and are presently a topic of significant discussion associated with liver injury.[3] Excessive consumption of ethanol can cause a series of hepatic injuries, contributing to the development of alcoholic

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

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liver diseases, which are characterized by fat accumulation and inflammation in the liver, leading to cirrhosis and hepatocellular carcinoma.[4] Progression of alcoholic liver diseases is a multifactorial process, which involves a number of genetic, nutritional, and environmental factors.[5] Presently, there is substantial evidence that oxidative stress is involved in the pathogenesis and progression of liver injuries.[6] Ethanol intake increases the production of free radicals or reactive oxygen species and causes oxidative stress by compromising the antioxidant defense system.[7]

Glutathione S-transferases (GSTs) are widely used Phase II drug-metabolizing enzymes that have the function of detoxification in humans and animals. They are the most important part of the antioxidant defense system in an organism, contributing to the resistance against attacks of electrophilic substances and catalyzing the reaction of exogenous chemicals to protect organisms.[8] GSTs are usually divided into eight superfamilies, alpha (A), kappa (K), mu (M), pi (P), sigma (S), theta (T), zeta (Z), and omega (O), where the dominant component in the human liver is alpha class [alpha-GST (GSTA)].[9] GSTA is encoded by a P12 gene cluster located in chromosome 6, which contains five subunits (GSTA1−A5). GST alpha 1 (GSTA1) is a predominant member of the GSTA family[10] and accounts for 65−75% of the total GSTs in the human liver. It plays an important role in the antioxidative defense system, which can catalyze many xenobiotics such as carcinogens, environmental toxins, and certain drugs; it can also combine with glutathione (GSH), promoting its degradation in the cells to remove and protect the body.[11] With some efficacy, therefore, GSTA1 can be used as a marker of liver injury. The changes of GSTA1 can be detected at a low level during the early stage of acute hepatic injury, and GSTA1 is a more sensitive and accurate indicator than alanine aminotransferase (ALT).[12]

The present study aimed to investigate whether SN actually has hepatoprotective effects against ethanol-induced hepatic injury *in vivo* and *in vitro,* and further explore the role of GSTA1 in liver detoxification as adjusted by SN. Regulation of GSTA1 might aid in the prevention of hepatopathy and the development of new drugs.

2. Methods

2.1. Reagents

The ethanol used in our study was purchased from the Shanghai Chemical Reagent Factory (Shanghai, China). Type IV collagenase, dimethyl sulfoxide, insulin, transferrin, heparin, dexamethasone, and trypan blue were purchased from the Sigma Chemical Co. (St Louis, MO, USA). GSTA1 detection kit was purchased from American Rapidbio Company (RB; 23830 Arminta Street, West Hills, CA 91304, USA). SN and silymarin were purchased from Harbin Jiacheng Dispensary (Harbin, China). The detection kits of ALT, aspartate aminotransferase (AST), malondialdehyde (MDA), superoxide dismutase (SOD), GSH, and glutathione peroxidase (GSH-Px) were obtained from the Nanjing Jiancheng Institute of Biotechnology (Nanjing, China).

2.2. Primary hepatocyte extraction and culture

The liver of mice was rinsed and digested by the use of the inverse perfusion method improved on Seglen two-step perfusion *in situ*. After washing three times with Hank's balanced salt solution, the liver was put into emundans culture medium to end the digestion and extract cells gently with tweezers. The hepatocytes were mixed fully with adherent culture medium after filtration and centrifugation (500 rpm, 3 minutes). The number and survival rate of hepatocytes were calculated after a trypan blue dyeing experiment. The hepatocytes were seeded to a culture plate containing 24 wells (5×10^4 cells/well) with rat tail collagen under the conditions of 5% CO_2 and 37°C for 6 hours, and then the nonadherent hepatocytes were discarded along with the culture fluid. The adherent culture medium was replaced with a growing culture medium without serum, after which the growing culture medium was substituted with the same culture medium after 24 hours.

2.3. Animals and treatment

Adult male Kunming mice (18−22 g body weight) were obtained from the Central Laboratory of Harbin Pharmaceutical Group Co., Ltd (Harbin, Heilongjiang Province, China). The animals were housed in a controlled environment under standard conditions at a temperature of 20 ± 2°C and a relative humidity of 40−60%; they were allowed free access to food (standard mice pellets) and water, and were acclimatized for at least 1 week prior to use. All procedures involving animals complied with the China National Institutes of Healthy Guidelines for the Care and Use of Laboratory Animals.

2.4. Preparation of SN aqueous extracts

The powder of SN (800 g) was dipped into 5000 mL water for 30 minutes, and then extracted continuously for 40 minutes at 100°C to remove most of the colored materials, oligosaccharides, and small-molecule compounds. The supernatant was thereafter concentrated using a rotatory evaporator at 90°C and dried by a vacuum drying oven at 70°C to afford SN aqueous extracts 185 g. The SN aqueous extracts accounted for 23% of the original herb of *Solanum nigrum.*

2.5. Hepatoprotective activities against ethanol-induced cytotoxicity

The hepatocytes were randomly divided into five groups ($n = 6$): control group, ethanol model group, high-concentration group of SN aqueous extracts (SN-H, 100 μg/mL), middle-concentration group of SN aqueous extracts (SN-M, 75 μg/mL), and low-concentration group of SN aqueous extracts (SN-L, 50 μg/mL), respectively. The control and ethanol model groups were treated with an equal volume of culture medium. After 12 hours, we replaced the culture medium, and each group was administered the culture medium with 100 mmol/L ethanol except for the control group. After

8 hours, the supernatant was collected for the determination of ALT, AST, and GSTA1; additionally, the hepatocytes were collected for the determination of MDA, SOD, and GSH.

2.6. Hepatoprotective activities against ethanol-induced acute hepatic injury

Forty-eight mice were randomly divided into six groups ($n = 8$): control group, ethanol model group, high-dose group of SN aqueous extracts (SN-H, 200 mg/kg), middle-dose group of SN aqueous extracts (SN-M, 150 mg/kg), low-dose group of SN aqueous extracts (SN-L, 100 mg/kg), and positive drug group, respectively. The positive drug group was administered 200 mg/kg silymarin, while the control and ethanol model groups were given an equal volume of physiological saline. Each group received the appropriate vehicle daily by gavage for 7 days, and then was administered 50% ethanol (14 mL/kg body weight) except for the control group. After 8 hours, the serum was collected for the determination of ALT, AST, and GSTA1, and the liver tissues were collected for determination of MDA, SOD, GSH, GSH-Px, and GSTA1. Subsequently, the liver tissues were preserved in 10% formalin for histopathological analysis.

2.7. Determination of indexes

ALT, AST, MDA, SOD, GSH, and GSH-Px were determined using detection kits according to the manufacturer's instructions.

2.8. Determination of GSTA1 content by enyzyme-linked immunosorbent assay

GSTA1 content was detected using the Mouse Glutathione S Transferase Alpha1 (GSTA1) ELISA Kit (American Rapidbio Company), which used Purified Mouse GSTA1 to coat microtiter plate wells, to make a solid-phase antibody. The procedure was routinely performed according to the manufacturer's instructions. The antibody had been diluted 500-fold and the liver samples were diluted fivefold. According to the reported method, the content of GSTA1 was determined by the color reaction that produces a yellow complex with maximum absorption at 450 nm. A quantitative measure of GSTA in the sample was calculated using the generated standard curve, and the limits of quantification were determined to be 0.7−125 ng/mL. All values were normalized by the total protein concentration of the same sample.

2.9. Histopathological analysis

Formalin-fixed specimens were embedded in paraffin and sectioned at 5 μm thickness, according to the routine procedure. After hematoxylin and eosin staining, the slides were observed for conventional morphological evaluation under a light microscope and photographed at 400× magnification.

2.10. Statistical analysis

Values were expressed as mean ± standard deviation, and statistical significance was determined by one-way analysis of variance using the SPSS software 19.0 (SPSS, Inc., Chicago, IL, USA). The Tukey's multiple comparison test in *post hoc* multiple comparisons was used to examine the statistical significance ($p < 0.05$ and $p < 0.01$) between groups.

3. Results

3.1. Protective effects of SN against ethanol-induced hepatic injury in vitro

3.1.1. ALT and AST activities in hepatocyte culture supernatant

Compared with the control group, ALT and AST activities in the model group increased significantly ($p < 0.01$), which indicated that the model was replicated successfully. When treated by the high-concentration SN aqueous extracts (100 μg/mL), ALT and AST activities decreased markedly ($p < 0.05$) compared with the model group. The results of ALT and AST activities in hepatocyte culture supernatant are presented in Fig. 1.

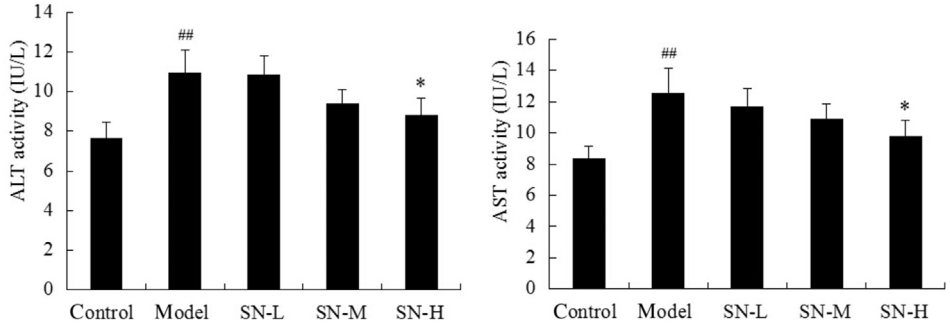

Fig. 1. Changes in ALT and AST activities in hepatocyte culture supernatant. Values are expressed as mean ± SD in each group; $n = 6$. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ compared to the model group. $^{*}p < 0.05$ and $^{**}p < 0.01$ compared to the control group. ALT = alanine aminotransferase; AST = aspartate aminotransferase; Control = control group; Model = ethanol model group; SD = standard deviation; SN-H = group administered with *S. nigrum* at a dose of 100 μg/mL; SN-L = group administered with *S. nigrum* aqueous extracts at a dose of 50 μg/mL; SN-M = group administered with *S. nigrum* aqueous extracts at a dose of 75 μg/mL. The significance of * in the footnotes means comparing with the control group and # means comparing with the model group.

3.1.2. MDA, SOD, and GSH in hepatocytes

MDA, SOD, and GSH in the model group were significantly different ($p < 0.01$) from those of the control group, which indicated that the model was replicated successfully. When treated by the high-concentration SN aqueous extracts (100 μg/mL), MDA decreased markedly ($p < 0.05$), and SOD and GSH increased markedly ($p < 0.05$) compared with the model group. The results of MDA, SOD, and GSH in hepatocytes are presented in Table 1.

3.1.3. GSTA1 content in hepatocyte culture supernatant

The GSTA1 content of the model group increased significantly ($p < 0.01$) compared with that of the control group, which indicated that the model was successfully replicated. Compared with the model group, however, GSTA1 content decreased markedly ($p < 0.05$) in SN-M and significantly ($p < 0.01$) in SN-H, which was more sensitive than ALT and AST ($p < 0.05$). When treated by the high-concentration SN aqueous extracts (100 μg/mL), the GSTA1 content was close to the control group and consistent with other detection indexes. The results of GSTA1 content in hepatocyte culture supernatant are presented in Fig. 2.

3.2. Protective effects of SN against ethanol-induced hepatic injury in vivo

3.2.1. ALT and AST activities in the serum

Compared with the control group, ALT and AST activities of the model group increased significantly ($p < 0.01$), which indicated that the model was replicated successfully. Serum ALT and AST activities in SN-M and SN-H decreased significantly ($p < 0.01$) compared with those in the model group. The prevention effects were almost the same as that in the positive drug group with a high dose of SN aqueous extracts, 200 mg/kg, in treated animals. The results of ALT and AST activities in the serum are presented in Fig. 3.

Table 1
Changes in MDA, SOD, and GSH in primary hepatocytes.

Group	MDA	SOD	GSH
	(nmol/mg protein)	(U/mg protein)	(μmol/g protein)
Control	1.05 ± 0.23	23.71 ± 2.67	8.64 ± 1.07
Model	1.97 ± 0.15$^{\#\#}$	16.90 ± 1.44$^{\#\#}$	5.98 ± 0.83$^{\#\#}$
SN-L	1.89 ± 0.14	17.57 ± 0.94	6.32 ± 0.80
SN-M	1.69 ± 0.13	19.09 ± 1.27	6.98 ± 0.65
SN-H	1.58 ± 0.12*	20.97 ± 2.06*	7.81 ± 0.99*

Values are expressed as the mean ± SD in each group; $n = 6$.
$^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ compared to the model group.
$^{*}p < 0.05$ and $^{**}p < 0.01$ compared to the control group.
Control = control group; GSH = glutathione; MDA = malondialdehyde; Model = ethanol model group; SD = standard deviation; SN-H = group administered with *S. nigrum* at a dose of 100 μg/mL; SN-L = group administered with *S. nigrum* aqueous extracts at a dose of 50 μg/mL; SN-M = group administered with *S. nigrum* aqueous extracts at a dose of 75 μg/mL; SOD = superoxide dismutase.

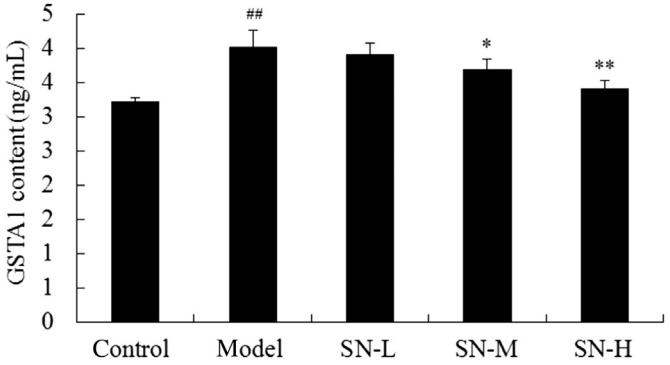

Fig. 2. Changes in GSTA1 content in hepatocyte culture supernatant. Values are expressed as mean ± SD in each group; $n = 6$. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ compared to the model group. $^{*}p < 0.05$ and $^{**}p < 0.01$ compared to the control group. Control = control group; GSTA1 = glutathione S-transferase alpha 1; Model = ethanol model group; SD = standard deviation; SN-H = group administered with *S. nigrum* at a dose of 100 μg/mL; SN-L = group administered with *S. nigrum* aqueous extracts at a dose of 50 μg/mL; SN-M = group administered with *S. nigrum* aqueous extracts at a dose of 75 μg/mL.

3.2.2. MDA, SOD, GSH, and GSH-Px in the liver

MDA, SOD, GSH, and GSH-Px of the model group were significantly different ($p < 0.01$) from those of the control group, which indicated that the model was reproduced successfully. Compared with the model group, MDA decreased markedly in SN-M ($p < 0.05$) and SN-H ($p < 0.01$), whereas SOD, GSH, and GSH-Px increased markedly ($p < 0.05$) in SN-M and decreased significantly ($p < 0.01$) in SN-H. The prevention effects were almost the same as that in the positive drug group, with a high dose of SN aqueous extracts, 200 mg/kg, in treated animals. The results of MDA, SOD, GSH, and GSH-Px in the liver are presented in Table 2.

3.2.3. GSTA1 contents in the serum and liver

GSTA1 contents in the serum and liver in the model group were significantly different ($p < 0.01$) from those in the control group, which indicated that the model was reproduced successfully. Serum GSTA1 in SN-M and SN-H decreased significantly ($p < 0.01$) compared with the model group (Fig. 4A). Liver GSTA1 increased markedly ($p < 0.05$) in SN-M and decreased significantly ($p < 0.01$) in SN-H (Fig. 4B). The prevention effects were almost the same as that in the positive drug group with a high dose of SN aqueous extracts, 200 mg/kg, in treated animals, which was consistent with other detection indexes. The results of GSTA1 contents in the serum and liver are presented in Fig. 4.

3.2.4. Histopathological analysis

The round vacuoles, inflammatory cell infiltration, and hepatocyte spotty necrosis are shown in the ethanol model group. The slight inflammation of hepatocytes in the positive drug group is reduced, and the hepatic lobules are more clear and complete than those in the model group. The degree of injury of the high-dose group of SN aqueous extracts, 200 mg/kg, is obviously lower than that of the model group, which is close to the positive drug group.

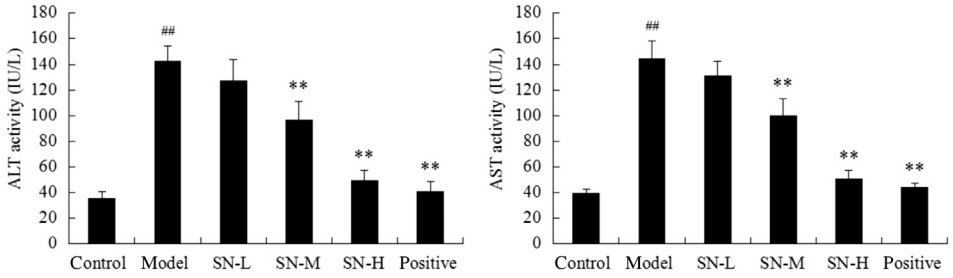

Fig. 3. Changes in ALT and AST activities in the serum of mice with ethanol-induced acute hepatic injury. Values are expressed as mean ± SD in each group; $n = 8$. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ compared to the model group. $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with the control group. ALT = alanine aminotransferase; AST = aspartate aminotransferase; Control = control group; Model = ethanol model group; Positive = group administrated with silymarin at a concentration of 200 mg/kg; SD = standard deviation; SN-H = group administered with *S. nigrum* at a dose of 100 μg/mL; SN-L = group administered with *S. nigrum* aqueous extracts at a dose of 50 μg/mL; SN-M = group administered with *S. nigrum* aqueous extracts at a dose of 75 μg/mL.

Table 2
Changes in MDA, SOD, GSH, and GSH-Px in the liver.

Group	MDA	SOD	GSH	GSH-Px
	(nmol/mg protein)	(U/mg protein)	(μmol/g protein)	(U/mg protein)
Control	6.96 ± 1.03	92.47 ± 8.34	14.97 ± 1.15	310.98 ± 31.62
Model	10.45 ± 0.88##	59.87 ± 9.32##	9.52 ± 1.87##	197.88 ± 26.82##
SN-L	9.88 ± 0.50	63.90 ± 5.64	10.15 ± 1.16	207.27 ± 24.09
SN-M	8.70 ± 0.63*	77.49 ± 4.41*	12.96 ± 0.97*	255.53 ± 40.54*
SN-H	7.88 ± 0.55**	84.74 ± 5.23**	13.78 ± 1.07**	299.60 ± 34.73**
Positive	7.31 ± 0.93**	89.89 ± 9.46**	14.09 ± 1.74**	302.18 ± 27.78**

Values are expressed as the mean ± SD in each group; $n = 8$.
$^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ compared to the model group.
$^{*}p < 0.05$ and $^{**}p < 0.01$ compared to the model group.
Control = control group; GSH = glutathione; GSH-Px = glutathione peroxidase; MDA = malondialdehyde; Model = ethanol model group; Positive = group administrated with silymarin at a concentration of 200 mg/kg; SD = standard deviation; SN-H = group administered with *S. nigrum* at a dose of 100 μg/mL; SN-L = group administered with *S. nigrum* aqueous extracts at a dose of 50 μg/mL; SN-M = group administered with *S. nigrum* aqueous extracts at a dose of 75 μg/mL; SOD = superoxide dismutase.

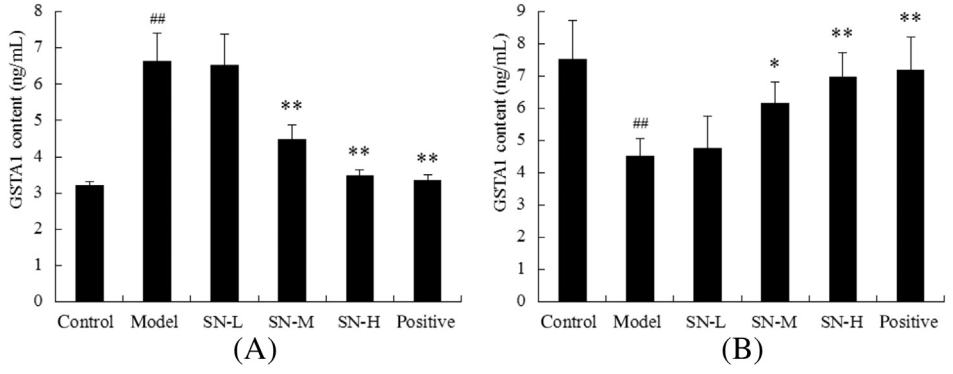

Fig. 4. Changes in GSTA1 contents in the (A) serum and (B) liver of mice with ethanol-induced acute hepatic injury. Values are expressed as mean ± SD in each group; $n = 8$. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ compared to the model group. $^{*}p < 0.05$ and $^{**}p < 0.01$ compared to the control group. Control = control group; GSTA1 = glutathione S-transferase alpha 1; Model = ethanol model group; Positive = group administrated with silymarin at a concentration of 200 mg/kg; SD = standard deviation; SN-H = group administered with *S. nigrum* at a dose of 100 μg/mL; SN-L = group administered with *S. nigrum* aqueous extracts at a dose of 50 μg/mL; SN-M = group administered with *S. nigrum* aqueous extracts at a dose of 75 μg/mL.

4. Discussion

SN is one of the medicinal herbs that belong to the family "Solanaceae" and has been used traditionally to treat various ailments.[13] A glycoprotein isolated from SN has been shown to have antioxidative activity[14]; antioxidant activity was shown to be correlated to polyphenol content, signifying that phenolic compounds are probably responsible for this property. Hence, the main mode of conferred hepatoprotection appears to be through control of oxidative damage and free

radical scavenging activity.[15] The whole plant of SN is antiperiodic, antiphlogistic, diuretic, emollient, febrifuge, narcotic, purgative, and sedative.[16] In this experiment, we selected SN as a preventive drug and studied its hepatoprotective effects, using primary hepatocytes and animal models challenged with ethanol.

Previous findings by our group have established three acute hepatic injury models induced by carbon tetrachloride, acetaminophen, and ethanol in mice, and have proved that GSTA1, one of the main Phase II drug-metabolizing enzymes and that which can catalyze many xenobiotics, was detected at low concentrations during the early stages of acute hepatic injury. Thus, GSTA1 is a more sensitive and more accurate indicator than ALT,[10] and the changes of GSTA1 in this study provide further evidence supporting this viewpoint.

Primary hepatocytes are the golden standards for studying hepatotoxicity of drugs *in vitro*,[17] which maintain both Phase I and Phase II metabolic activities as well as uptake transporter activity.[18] Unlike immortalized cell lines, freshly isolated primary hepatocytes *in vitro* retain liver-specific functions related to drug metabolism.[19] In this experiment, primary hepatocytes were obtained from the liver of mice by the inverse perfusion method improved on Seglen two-step perfusion *in situ*. Three doses of SN aqueous extracts were treated in the primary hepatocytes to study the hepatoprotective effects. In the presence of SN aqueous extracts (100 μg/mL), no hepatocytic damage was observed in the cells treated with ethanol, compared with the model group; GSTA1 was more sensitive than ALT and AST. These results are consistent with our previous findings. In primary hepatocytes, with a treatment does, 100 mg/mL, SOD activity and GSH content increased markedly, while MDA content decreased markedly, which suggested that no significant cytotoxic effects were found at a high dose of SN. Therefore, SN plays an important role in antioxidant activity and also has the effect of reducing lipid peroxidation injury. Thus, we estimated that SN has obvious hepatoprotective effects against ethanol-induced injury at a cellular level and the optimal dose is 100 μg/mL.

An acute hepatic injury model induced by ethanol in mice was replicated successfully according to our previous studies. Three doses of SN aqueous extracts were given to the animals, using silymarin as a reference, which is an antihepatotoxic substance that has been investigated as a supportive treatment for liver damage from various chemicals or toxins.[20] *In vivo*, mice that received SN aqueous extracts (150 mg/kg) with ethanol showed marked prevention of ethanol-induced hepatotoxicity, as evidenced by the significant reductions of serum transaminases (ALT and AST), and variation of hepatic oxidative indexes (MDA, SOD, GSH, and GSH-Px) and GSTA1 compared with the model group. Furthermore, for mice that received SN aqueous extracts (200 mg/kg), all the detection indexes were significantly different from those of the model group, and the prevention effects were almost the same as those in the positive drug group. These results suggested that SN can remove harmful free radicals, block the magnified chain reaction of free radicals, and maintain hepatocellular redox homeostasis, thus protecting the integrity of hepatocytes. Consequently, we estimated that SN has obvious hepatoprotective effects against ethanol-induced acute injury in mice and the optimal dose is 200 mg/kg.

GSTA1 is highly expressed in the normal liver,[21] and its protein plays an important role in the body's antioxidant system, which catalyzes the conjugation of GSH with carcinogens, drugs, toxins, or products of oxidative stress.[22] In this study, animals were administered SN, whereafter serum GSTA1 content decreased significantly at doses of 150 mg/kg and 200 mg/kg, while liver GSTA1 content increased significantly at doses of 150 mg/kg and 200 mg/kg. These results indicated that SN has obvious hepatoprotective effects against ethanol-induced acute injury. Simultaneously, it protects the integrity of hepatocytes and thus reduces the release of liver GSTA1, which contributes to an improvement in liver detoxification. Noticeably, the administration of SN at a dose of 200 mg/kg/d almost prevented liver dysfunctions from alcoholic toxicity.

In conclusion, the changes of all the indexes suggested that GSTA1 is a more sensitive indicator of hepatotoxicity. SN has hepatoprotective effects against ethanol-induced hepatic injury both *in vitro* and *in vivo*, and it may be an important free radical scavenger of hepatocytes, protecting the integrity of hepatocytes to reduce the release of liver GSTA1; then GSTA1 could participate in the oxidative stress and protect the liver against injuries. Therefore, GSTA1 plays an important role in preventing liver dysfunction from alcoholic toxicity, and the regulation of GSTA1 might aid in the prevention of hepatopathy and development of new drugs.

Acknowledgments

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