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Protective effect of *Solanum Nigrum* Linn green fruit ethanolic extract on alcoholic liver injury in mice

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Abstract Alcoholic liver injury is a liver disease caused by excessive alcohol consumption, which can lead to chronic liver disease death. *Solanum Nigrum* Linn taste bitter, cold, has the effect of clearing heat and detoxification, promoting blood and detumescence. *Solanum Nigrum* Linn fruit contains a variety of antioxidant enzymes, can remove the body produced by aerobic metabolism harmful substances. In this paper, a model of alcohol-induced liver injury in C57BL/6 mice was established to evaluate the protective effect of *Solanum Nigrum* Linn green fruit (SNGF) ethanolic extract on alcohol-induced liver injury. H&E staining and oil red O (ORO) staining showed that hepatic lobules were clearly demarcated, vacuoles were significantly reduced and lipid droplets were reduced in SNGF ethanolic extract treatment group. Serum levels of TC, TG, LDH, TBA, AKP, ALT and AST were decreased in the SNGF ethanolic extract treatment group, and SNGF ethanolic extract could clear reactive oxygen species (ROS) in time. MDA content was significantly decreased after SNGF ethanolic extract treatment, while superoxide dismutase (SOD) and GSH-Px contents were increased after SNGF ethanolic extract treatment. These results suggest that SNGF ethanolic extract has a protective effect on alcohol-induced liver injury.

Keywords: *Solanum Nigrum* Linn green fruit ethanolic extract; alcoholic liver injury; protective effect

1 Introduction

Alcoholic liver injury is one of the major causes of chronic liver disease death. The main reason is that acetaldehyde produced by alcohol dehydrogenase metabolism in liver cells can promote the production of reactive oxygen species (ROS) [1], which can induce oxidative stress response, destroy the body's oxidative balance and produce inflammatory factors [2, 3], thus affecting protein function and DNA damage recovery. Lipid accumulation and inflammatory response are induced, resulting in varying degrees of liver injury [4].

Solanum Nigrum Linn is an annual herb of the genus *Solanum* in the *Solanaceac* family. It is widely distributed and is a common medicinal and edible plant. *Solanum Nigrum* Linn is bitter and cold in taste, and has the effect of clearing heat and detoxification, promoting blood circulation and reducing swelling. The fruit can be picked when ripe in autumn [5]. *Solanum Nigrum* Linn fruit contains a variety of antioxidant enzymes, which can remove ROS and other harmful substances produced by aerobic metabolism in the body and have a protective effect on the body [6, 7]. At present, most studies on *Solanum Nigrum* Linn focus on its whole plant, leaves or ripe berries, and relatively few studies on the biological activity and mechanism of its unripe fruit [8]. This

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research group used network pharmacology and molecular docking technology to study the relationship between *Solanum Nigrum* Linn green fruit (SNGF) and alcoholic liver injury in the previous stage, which showed that SNGF contains a large number of polyphenol components [9].The aim of this study was to study the protective effect of SNGF ethanolic extract on alcoholic liver injury in mice, and to provide theoretical basis for prevention and treatment of liver injury.

2 Materials and methods

2.1 Materials and reagents

SNGF was collected in Shenyang, China. The fruit was extracted with 65% ethanol solution (1:10, μg/L), ultrasonically extracted at 40 °C for 30 min, then compressed and concentrated, extracted with n-butanol solution, vacuum evaporated, and preserved at 4 °C away from light.

2.2 Animal experimental design

Thirty-two male C57BL/6 mice, weighing about 20−25 g, were purchased from Liaoning Changsheng Biotechnology Co., LTD., and acclimated to the environment in the Laboratory Animal Center of Shenyang Pharmaceutical University for a week, during which they were free to drink and eat. The clean room temperature is 21−25 ℃, the humidity is 40%−60%, and the light time is 12 h/d. The routine operation of animal experiments is in accordance with the "Regulations on the Administration of Experimental Animals", and the license number of animal experiments is SYPU-IACU-S2022-11.30-104.

The mice were randomly divided into 4 groups with 8 mice in each group: Control group (CON group); Model group (EtOH group); EtOH and SNGF ethanolic extract group (EtOH + SNGF group); EtOH + Silibinin group. Mice in all groups were given Liebere-Decarli control liquid diet. After 5 days of acclimation, the control group and EtOH group were given pure water, and the EtOH + SNGF and EtOH + Silibinin groups were given 100 mg/kg SNGF ethanolic extract and Silibinin, respectively. The control group was given Lieber-DeCarli control liquid diet, and the other groups were given Lieber-DeCarli alcohol liquid diet containing 5% alcohol for 10 days. 45% dextrin solution and 31.5% (*V*/*V*) alcohol solution were prepared. On the 16th day, 45% dextrin solution was given to the control group, and 31.5% (*V*/*V*) alcohol solution was given to the other groups. The intragastric volume μL was body weight (g) × 20. After 9 h of instillation, the mice were killed by neck after taking blood from the orbit, and the livers were dissected. The blood was soaked and rinsed in 0.9% normal saline, and the surface moisture was dried with filter paper. The livers were weighed and photographed to observe the morphology of the livers. Part of the liver was fixed with formalin and part was frozen at −80 ℃ for later use.

2.3 Histopathological evaluation of liver

After the liver tissue was stripped, it was fixed with 4% paraformaldehyde for 2 h and denatured. After dehydration with gradient ethanol and xylene, it was embedded in paraffin wax. After curing, it was cut into liver cross-sectional sections with a thickness of 5 μm for subsequent pathological staining.

2.3.1 Hematoxylin and eosin (H&E) staining

The liver sections were dewaxed with xylene for three times, 10 min each time; the concentration from anhydrous ethanol to 90% and then to 80% was dehydrated with gradient ethanol for 5 min each; after rinsing with running water for 15 min, the concentration was stained with hematoxylin for 5 min; then the differentiation liquid was differentiated and the ammonia water was reverted to blue; after the eosin dye was redyed for 2 min, the gradient ethanol was dehydrated twice each. The tablets were sealed with xylene transparent neutral gum for 5 min each time. The tissue morphology after staining was observed under 20 and 40 times optical microscope and photographed.

2.3.2 Oil red O (ORO) stain

The liver sections were first dewaxed with xylene three times for 10 min each time, then dehydrated by gradient from anhydrous ethanol to 90% and then to 80% for 5 min each, rinsed with running water for 15 min, and soaked with 60% isopropyl alcohol for 20−30 s. Dye ORO working solution for 10 min. 60% isopropyl alcohol differentiated 5 s. Rinse with distilled water, dye the nucleus with hematoxylin staining solution for 1−2 min, rinse with distilled water, filter paper absorb the surrounding water, and seal the sheet with neutral glue. The tissue morphology after staining was observed under 20 and 40 times optical microscope and photographed.

2.4 Detection of serum biochemical indexes in mice

Orbital blood was collected in a 1.5 mL centrifuge tube and placed in a centrifuge for 30 min at a temperature of 4 ℃, a time of 15 min and a speed of 4 000 r/min. The upper layer of serum was absorbed and placed in a 1.5 mL centrifuge tube and refrigerated at 4 ℃. The contents of total cholesterol (TC), triglyceride (TG), lactate dehydrogenase (LDH), total bile acid (TBA), alkaline phosphatase (AKP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were detected according to the kit instructions.

2.5 Detection of ROS and antioxidant indexes in mouse liver tissue

The ROS content was detected by DHE probe. Fluorescence signal intensity of 2, 7-dichlorofluorescein (DCF) was determined by fluorescence microscope. The rinsed liver tissue was made into 10% tissue homogenate by adding 0.9% normal saline at 9 times the amount, centrifuged at 12 000 rpm for 10 min at low temperature, and the supernatant was taken as the sample to be measured. The contents of superoxide dismutase (SOD), malondialdehyde (MDA) and glutathione peroxidase (GSH-Px) in liver tissue were detected according to the instructions of the kit.

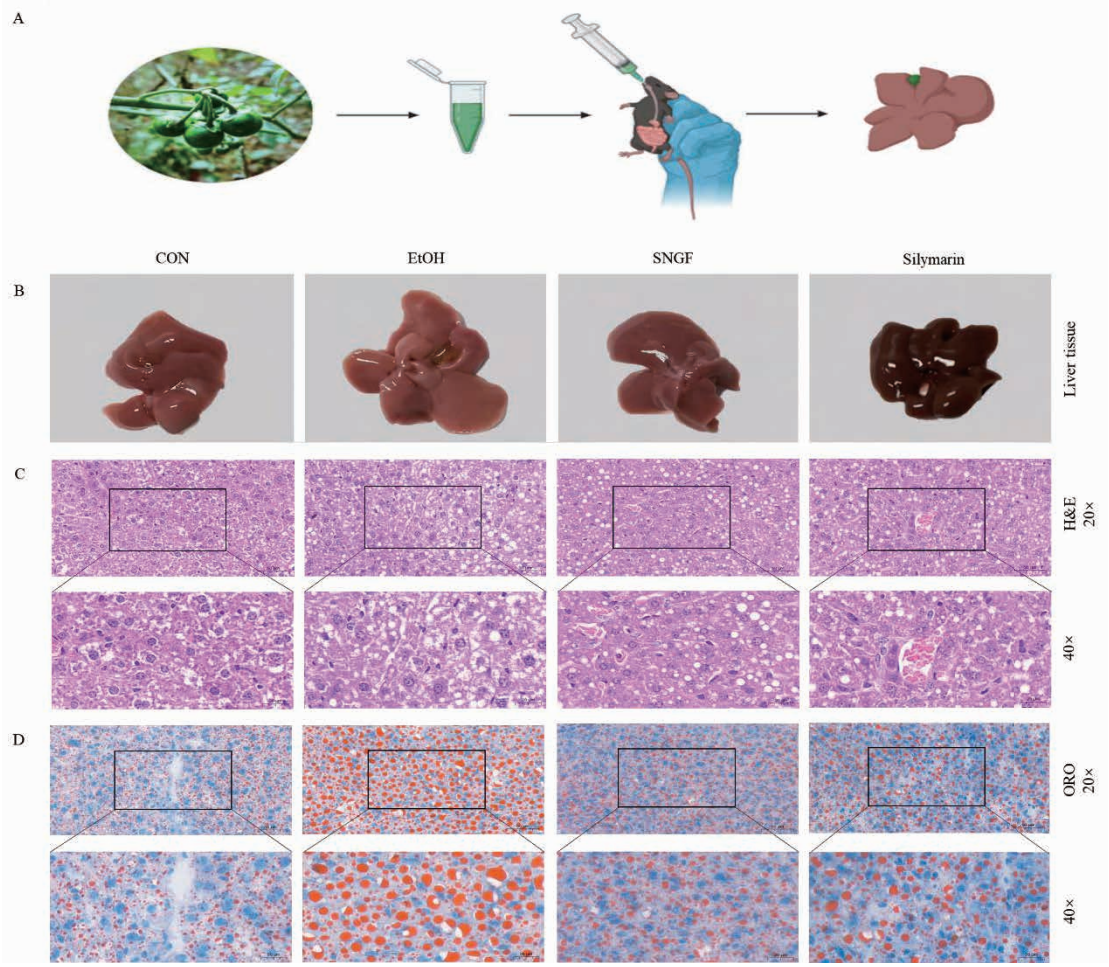
3 Results

3.1 Effects of SNGF ethanolic extract on alcoholic liver morphology in mice

Fig. 1A shows the schematic diagram of animal experiments. SNGF liquid extracted was given to mice by intragastric administration, and liver was taken after death. As shown in Fig. 1B, compared with the control group, the liver of mice in EtOH group was significantly enlarged, and the liver morphology was improved after SNGF ethanolic extract administration. H&E and ORO staining showed (Fig. 1C and D) that the liver tissue structure of normal mice was clear, hepatocytes were free of steatosis and red fat droplets, while the liver lobular structure of EtOH group mice was disordered, fat vacuolation was serious, fat droplets were present and large in volume. Compared with EtOH group, hepatic lobules in SNGF group were clearly demarcated, vacuoles were significantly reduced, and lipid droplets were reduced, suggesting that SNGF ethanolic extract had a protective effect on steatosis.

3.2 Effects of SNGF ethanolic extract on serum biochemical indices in mice

To further evaluate the protective effect of SNGF ethanolic extract against alcoholic fatty liver disease, some common biochemical indicators of fatty liver were detected in mouse serum. Compared with the control group, the serum levels of TC, TG, LDH, TBA, AKP, ALT and AST were significantly increased in the model group ($P < 0.001$), while the serum levels of TC, TG, LDH, TBA, AKP, ALT and AST were decreased in the SNGF ethanolic extract treatment group (Fig. 2 A−G). These results suggest that SNGF ethanolic extract treatment blocks the ability of alcohol to interfere with TC, TG, LDH, TBA, AKP, ALT and AST in mouse serum, suggesting that SNGF ethanolic extract may have a protective effect on alcohol-induced liver injury.

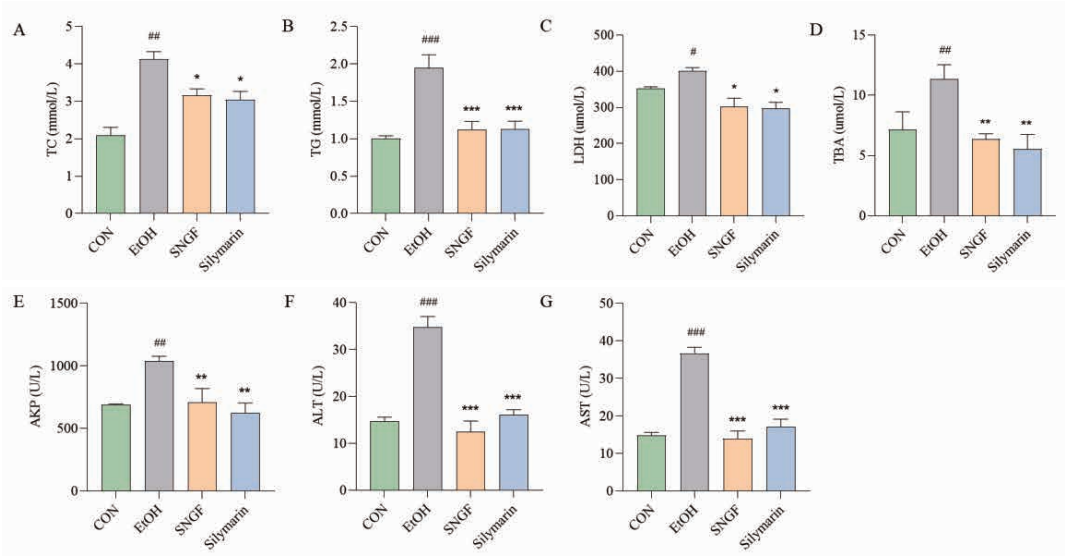


(A) − Animal experiments; (B) − Mouse liver morphology; (C) − H&E staining of liver sections; (D) − ORO staining of liver sections (Magnification: 20 ×, scale: 100 μm; 40 ×, scale: 50 μm).

Fig. 1 Animal experiments, liver histopathological images of liver morphology and liver sections

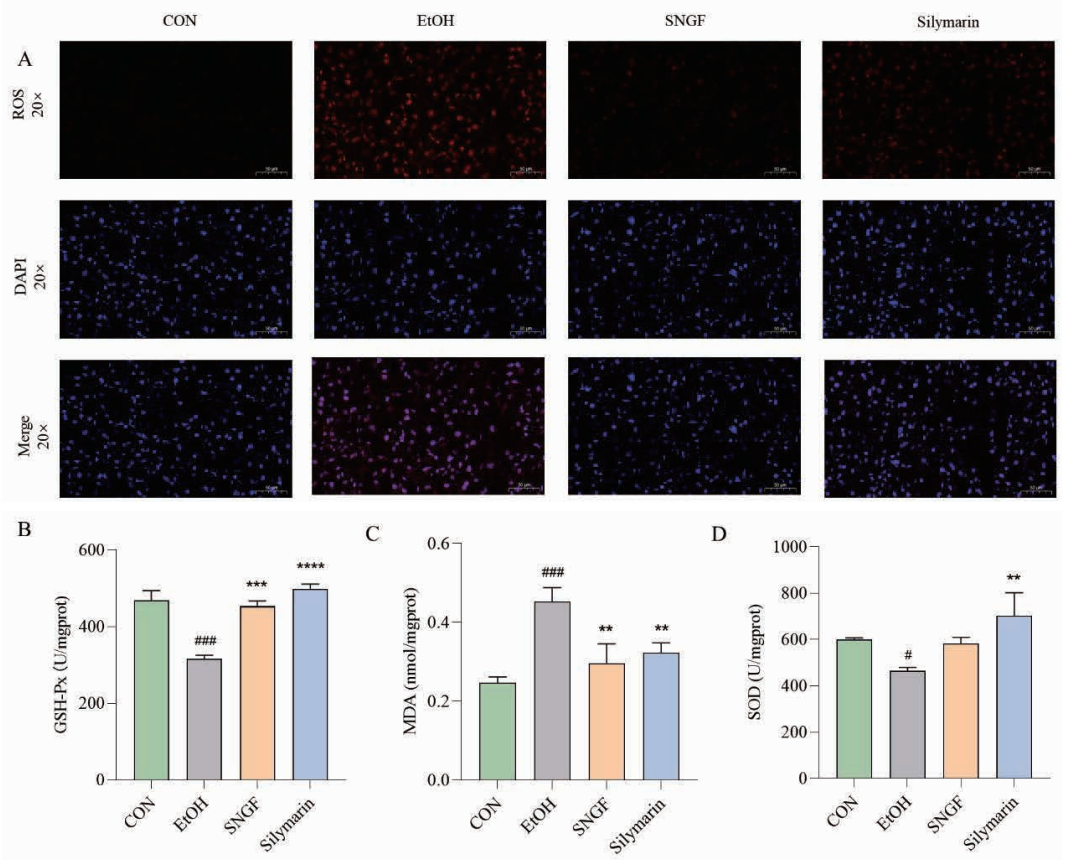
3.3 Effects of SNGF ethanolic extract on oxidative stress levels in alcoholic mice

In order to evaluate the effect of SNGF ethanolic extract on the antioxidant capacity, the activity of antioxidant enzymes was examined. As shown in Fig. 3A, SNGF ethanolic extract can remove ROS generated by the body in time, so that its generation and removal are in a state of dynamic balance. By detecting the activity of GSH-Px and the levels of SOD and MDA, the degree of lipid oxidative damage of the body or cells can be reflected, so as to reflect the level of oxidative stress of liver cells. As shown in Fig. 3C, compared with the control group, MDA levels in the liver of the model group were significantly increased ($P < 0.001$), and significantly decreased after SNGF ethanolic extract treatment. In contrast, the contents of SOD and GSH-Px in the liver of mice decreased significantly after feeding alcohol ($P < 0.01$), and increased after giving SNGF ethanolic extract (Fig. 3B, D). These results suggest that SNGF ethanolic extract can reduce lipid peroxidation and promote antioxidant capacity, thereby improving antioxidant defense.



The content of (A) – TC; (B) – TG; (C) – LDH; (D) – TBA; (E) – AKP; (F) – ALT; (G) – AST in serum; $n = 4$, mean ± SD; #: Compared with the control group, # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$;*: Compared to the alcohol group, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Fig. 2 Serum biochemical indices of mice



(A) – DHE fluorescence staining of liver sections; The content of (B) – GSH-Px; (C) – MDA; (D) – SOD in liver; $n = 4$, mean ± SD; #: Compared with the control group, # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$; *: Compared to the alcohol group, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Fig. 3 Effects of SNGF ethanolic extract on alcoholic oxidative stress in mice

4 Discussion

Solanum Nigrum Linn is distributed in all parts of China and has rich medicinal resources. The whole plant can be used as medicine to disperse blood stasis and reduce swelling, clear heat and detoxify, promote blood circulation, diuresis and reduce swelling [10]. *Solanum Nigrum* Linn is often used to treat diseases related to liver injury. Studies have confirmed that the alcohol extract of *Solanum Nigrum* Linn fruit can effectively reduce the levels of AST, ALT, ALP and total bilirubin in the rat model of acute liver injury induced by CCI4/CdCI2 [11, 12], and *Solanum Nigrum* Linn tablets have obvious therapeutic effects on primary liver cancer [13]. As a kind of ethnic medicine in Uyghur area of China, *Solanum Nigrum* Linn fruit is anti-inflammatory, bactericidal, helps to enhance the immune function of the human body, and can adjust the balance of body fluids [14]. The alkaloid content in the fruit of *Solanum Nigrum* Linn is more abundant than that in the commonly used dried whole grass parts in clinical practice, which indicates that nightshade has better fruit effect when it is used as medicine [15]. The content of steroid alkaloids in immature green berries of *Solanum Nigrum* Linn was higher than that of the whole plant, and its concentration and absolute content would gradually decrease with fruit ripening [16], but it may not be an effective component of the fruit to play an anti-inflammatory role. *Solanine* has the effect of anti-cancer nuclear division, and the total *solanine* extracted from dried green fruit of *solanine* has an inhibitory rate of 40%−50% on transplanted tumor in animals [17]. *Solanum Nigrum* Linn green fruit also has high contact activity against Diamondback moth [18]. In this paper, the mechanism effect of SNGF ethanolic extract on the protection of alcohol-induced C57BL/6 mouse liver injury was studied through the establishment of alcohol-induced liver injury model in mice, which provided early theoretical support for the prevention and treatment of liver injury and liver cancer by SNGF, and provided basis for clinical rational and safe drug use.

5 Conclusion

In conclusion, in this paper, alcohol-induced liver injury of C57BL/6 mice was used as the model, and SNGF ethanolic extract was used as the research object. By observing liver morphology, H&E staining and ORO staining were used to observe the pathological situation of liver tissues. The accumulation of liver fat droplets in liver tissues of SNGF group mice was reduced, and cell morphology and steatosis were significantly improved. These results indicate that SNGF ethanolic extract has protective effect on steatosis. By detecting the content of markers of alcoholic liver injury, it can be seen that SNGF ethanolic extract treatment blocks the interference ability of alcohol on TC, TG, LDH, TBA, AKP, ALT and AST in serum of mice. By detecting the level of markers of oxidative stress, SNGF ethanolic extract treatment can clear ROS in the body and significantly reduce the level of MDA. The increase of SOD and GSH-Px content indicates that SNGF ethanolic extract can promote antioxidant capacity and improve antioxidant defense capacity. It can be concluded that SNGF ethanolic extract has a protective effect on alcoholic liver injury.

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