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Effects of saponin extract from *Panax notoginseng* on hyperlipidemic mice

Zheng-yong Yu[1#], Ya-juan Luo[2#], Tai-hui Yang[3], Wen-bo Zeng[1], Heng Liu[3], Wen-feng Xin[1] and Guang-quan Feng[1]*

[1]Wenshan University–College of Notoginseng Medicine and Pharmacy, Yunnan, China
[2]Shanghai TCM-Integrated Hospital, Shanghai, China
[3]Dali University, Yunnan Provincial Key Laboratory of Entomological Biopharmaceutical, Yunnan, China

Abstract: To investigate the impact of the *Panax notoginseng* saponin extract on hyperlipidaemic mice. We developed a hyperlipidemia model in mouse through the administration of a high-fat diet. We conducted weekly measurements of body weight, serum total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C). Additionally, serum levels of Apolipoprotein A (APOA) and Apolipoprotein B (APOB) were determined post-feeding. We assessed pathological liver tissue damage in mice, as well as examined malondialdehyde (MDA) levels and superoxide dismutase (SOD) activity in liver tissue. Immunohistochemical analysis was conducted to detect the expression levels of heme oxygenase-1 (HO-1) and nuclear factor erythroid 2-related factor 2 (Nrf2) proteins within liver tissues. Administration of *Panax notoginseng* saponin extract led to a reduction in body weight, liver index, and histopathological scores among mice. Additionally, there was a significant reduction in TC, TG and LDL-C levels, accompanied by an increase in HDL-C levels. Additionally, an increase in hepatic SOD activity and a decrease in MDA content were observed in the liver homogenates of mice. Furthermore, the expression levels of HO-1 and Nrf2 proteins were upregulated in liver tissue. These findings suggest that *Panax notoginseng* saponin extracts may ameliorate high-fat diet-induced hyperlipidemia.

Keywords: Hyperlipidemia, saponin from *Panax notoginseng,* Nrf2/HO-1.

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INTRODUCTION

Hyperlipidemia (HLP), refers to the condition where one or more types of lipids in the plasma are higher than normal levels. It is caused by abnormalities in the metabolism or transport of fats (Stewart *et al*., 2020). As a result of unhealthy eating habits, smoking and alcohol consumption, the incidence of this condition has significantly increased, which is now affecting individuals at younger ages and posing a serious threat to human health. Atherosclerosis, myocardial infarction and stroke are associated with hyperlipidemia (Tabas and Bornfeldt, 2020). The management of hyperlipidemia often involves interventions such as improving dietary habits, increasing physical activity, and using medications. Currently, drugs commonly used to treat hyperlipidemia include statins, fibrates, and bile acid sequestrants (Newman, 2022; Cai *et al*., 2021). However, these medications may also induce various adverse reactions such as abdominal pain, dry mouth, gastrointestinal disturbances. The development of new treatment methods for hyperlipidemia is urgently needed.

As people's interest in traditional Chinese medicine grows, natural Chinese medicinal materials with dietary therapy functions have become a promising method for preventing and treating hyperlipidemia. Studies have found that improving dietary habits and increasing functional nutrients in food can effectively intervene in changes in blood lipid levels. *Panax notoginseng* is the dried root of the plant belonging to the family Araliaceae. It is known for its various medicinal properties, including hemostasis, blood tonifying, Relieving pain and swelling, promoting circulation and resolving stasis (Han *et al*., 2022). Research has shown that the mixture or single component of *Panax notoginseng* saponins has neuroprotective effects (Wang *et al*., 2024; Wu *et al*, 2020) cardiovascular protective effects (Chen *et al*., 2021; Yang *et al.*, 2014) immune system protective effects (Wu, 2022), lipid-lowering activity (Shin *et al*., 2021), anti-atherosclerotic activity (Yang *et al*., 2022) and Among them, the effect on the cardiovascular system is the most prominent (Zhang *et al*., 2018; Liu *et al*., 2019). However, there are many types of components in *Panax notoginseng* saponins and the yield of rare saponins (such as ginsenoside Rh4, Rk3, CK, etc.) with significant activity in the extract obtained by traditional extraction methods is low, which limits the further use of *Panax notoginseng* extract in the treatment process. Our research team used Cordyceps militaris to ferment *Panax notoginseng*, which increased the content of rare saponins in the extract and further improved the biological activity of *Panax notoginseng* saponin extract.

**Corresponding authors:* e-mails: 19969146492@163.com #These authors contributed equally to this work

Searching for safe and effective solutions is of great importance natural lipid-regulating biological resources for the prevention and treatment of hyperlipidemia. *Panax notoginseng* is a key component of many drugs and has important medicinal and economic value. Although there have been reports on its anti-hyperlipidemia effects, the pharmacological and molecular mechanisms of *Panax notoginseng*'s lipid-lowering effects have not been fully elucidated. Here, to investigate the effects of *Panax notoginseng* saponin extract on hyperlipidemic mice, we conducted a basic study of the substance by fermenting *Panax notoginseng* with Cordyceps militaris and constructed a mouse model of hyperlipidemia Providing them with a high-fat diet. We confirmed that *Panax notoginseng* saponin extract has a therapeutic effect on hyperlipidemia in mice.

MATERIALS AND METHODS

Chemicals and reagents

We purchased HPLC-grade acetonitrile from Thermo Fisher Scientific (China). There were several substances obtained from the National Food and Drug Administration, including notoginsenoside R1, ginsenoside Rg1, Rc, Rb2, Rb3, Rd, F2, Rk3, Rh4, 20(R)-Rg3, CK and Rh2. Chengdu Kelong Chemical Co., Ltd. (Chengdu, China) provided the ethanol. Cordyceps pruinosa Petch was obtained from the soil of *Panax notoginseng* and identified by Dr. Wenbo Zeng of Wenshan University Sanqi Medical College. In addition to the TG, TC, HDL, LDL cholesterol, super oxide dismutase and malondialdehyde (MDA) kits, Jiancheng Bioengineering Institute provided kits for potassium chloride, sodium chloride and phosphate ions. (Nanjin). Yuanju Biological Technology Center (Shanghai, China) provided apoprotein A (apoA) kit and apoprotein B (apoB) kit. Simvastatin was obtained from CSPC Pharmaceutical Group Co., Ltd. (Heb, China).

Drugs preparation

The preparation of the *Panax notoginseng* saponin extract was conducted as follows: A minimal quantity of Cordyceps pruinosa Petch mycelium was isolated from the mother culture and inoculated onto a Potato Dextrose Agar (PDA) solid medium. Following an incubation period of approximately 7 days at 25°C, the activated strain was transferred to a PDA liquid medium and subjected to agitation for an additional 7 days. Subsequently, approximately 20 grams of coarsely powdered *Panax notoginseng* main root was accurately weighed and incorporated into 33 milliliters of distilled water. The mixture was sterilized using high-pressure steam for 30 minutes, subsequently cooled and inoculated with 5 mL of the previously expanded culture broth. Over a 60-day period, the blend was uniformly stirred and incubated in the absence of light at a constant temperature of 25°C. Following incubation, the mixture was dried at 60°C and subsequently pulverized into a fine powder. A specific quantity of *Panax notoginseng* mycelium powder was then accurately weighed and subjected to extraction with 70% ethanol (v/v) through refluxing (gentle boiling) for 2.5 hours per cycle, with the process being repeated three times. *Panax notoginseng* saponin extract is obtained by concentrating the combined extract and removing alcohol odor. A suitable amount of extract was dissolved in pure water and extracted with n-butanol. The extract was concentrated to yield *Panax notoginseng* saponin extract.

HPLC analysis of the panax notoginseng saponin extract

Approximately 2-4 mg of *Panax notoginseng* saponin R1, along with ginsenosides Rg1, Rb1, Rc, Rb2, Rb3, Rd, F2, Rk3, Rh4, 20(R)-Rg3, CK and Rh2, were accurately measured and dissolved in a volumetric flask, which was subsequently adjusted to a final volume of 25mL. The resulting solution was filtered using a 0.45μm micropore membrane and reserved for subsequent analysis. Aliquots of 2μL, 4μL, 6μL, 8μL, 10μL, 15μL, 20μL, 25μL, 30μL, 35μL and 40μL of this control solution were then precisely pipetted and injected into a liquid chromatograph (Shimadzu Corporation, Japan) to construct a standard calibration curve. The analysis The experiment was conducted under controlled conditions, employing a mobile phase composed of acetonitrile and water with gradient elution. The acetonitrile concentration was adjusted as follows: from 0 to 12 minutes, 5%-18%; from 12 to 22 minutes, 18%-26%; from 22 to 30 minutes, 26%-35%; from 30 to 40 minutes, 35%-37%; from 40 to 48 minutes, 37%-45%; from 48 to 68 minutes, 45%-63%; and from 68 to 75 minutes, 63%-95%. The flow rate was maintained at 0.6mL/min, with a detection wavelength set at 203 nm and a column temperature of 30°C. The analysis was carried out using a Sepax Bio-C18 column (250mm x 4.6mm, 5μm, 200 A) supplied by Sepax Technologies. The regression equation, linear correlation coefficient and linear range were established by employing the peak area as the independent variable (x) and the standard injection volume (weight of the standard) as the dependent variable (y). A 10 μL injection of *Panax notoginseng* saponin extract, with a concentration of 66.7mg/mL, was introduced into the chromatograph under the specified conditions. The total saponin content was quantified by substituting the measured peak area value into the regression equation, thereby facilitating the determination of the saponin monomer content within the extract.

Animals

Male KM mice (n=48, weighing 20±2 g, Certificate No. SCXK (Xiang) 2019-0004) were procured from Hunan Anshengmei Pharmaceutical Research Institute Co., Ltd (Hunan, China). Following a one-week period, mice were housed in a climate-controlled facility (25°C, 50%

humidity, 12-hour light/dark cycle) with ad libitum access to food and water.

Grouping and Administration

A cohort of 48 healthy male KM mice, sourced from a single batch, was randomly allocated into various experimental groups for the study. These groups included a normal control group, a model group, a simvastatin group (10mg/kg) and groups receiving low (50mg/kg), medium (100mg/kg) and high (200mg/kg) doses of *Panax notoginseng* saponin extract, with each group comprising 8 mice. Over an initial 4-week period, all experimental groups, except for the normal control group, were subjected to a high-fat diet to induce hyperlipidemia, thereby establishing a mouse model for the condition. From the 5th to the 8th week, the control and hyperlipidemic model groups were given 0.9% saline orally, while the low, medium, and high dosage groups received *Panax notoginseng* saponin extract for 4 weeks. Body weight of all mice was monitored weekly to track changes over time.

Evaluation of body weight and liver index

Each week, the mice were weighed and subsequently euthanized to facilitate liver dissection and subsequent weighing. The liver index was calculated using the specified formula: liver index= $\frac{\text{Liver weight (g)}}{\text{mouse weight (g)}} \times 100$

Evaluation of SOD viability MDA content in liver

The liver tissue was combined with a saline solution in a 1:9 ratio and subsequently homogenized. Following the protocols outlined in the assay kits, the activities of superoxide dismutase (SOD) and malondialdehyde (MDA) were quantified in the liver samples.

Evaluation of serum biochemical indexes

Blood samples were procured from the medial canthus of mice at designated intervals throughout the study period. Subsequent to collection, serum was isolated and preserved for subsequent analysis. Prior to blood sampling, the mice were subjected to a 12-hour fasting period, during which they had ad libitum access to water following the final treatment. The mice were then euthanized via enucleation and blood samples were collected and centrifuged to separate the supernatant. These supernatants were subsequently employed to evaluate the concentrations of various biomarkers, including triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), apolipoprotein A (ApoA) and apolipoprotein B (ApoB), using commercially available kits.

Evaluation of liver HE staining

Initially, liver tissues were fixed for 48 hours in a 10% neutral formaldehyde solution. Subsequently, the tissues underwent gradient ethanol dehydration, xylene clearing, and paraffin embedding. The paraffin sections were then deparaffinized and rehydrated. Following this, the sections were stained with hematoxylin and eosin, dehydrated and sealed using neutral tree resin, xylene and anhydrous ethanol. The prepared slides were examined microscopically and images were captured for pathological analysis. The criteria for pathological scoring are outlined in table 1.

Evaluation of O staining in liver red oil

Frozen sections were rewarmed and dried. Subsequently, the samples were immersed in a 4% paraformaldehyde solution for 15 minutes, followed by rinsing with water and air-drying. The samples were then immersed in an oil red working solution for 8-10 minutes under light protection and gently differentiated in 75% alcohol. After washing with water, the samples were restained with hematoxylin for approximately 3-5 minutes, followed by rinsing in tap water. The sections were returned to blue using ammonia, washed again in tap water and the peripheral water was removed using filter paper. Finally, the sections were sealed with glycerol gel.

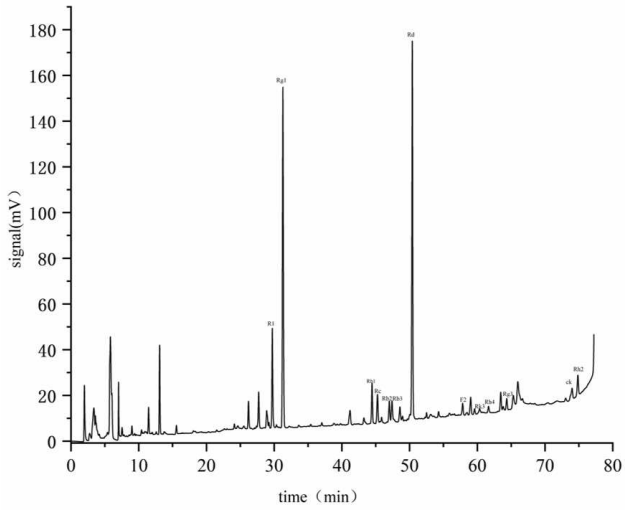

Fig. 1: HPLC chromatogram of *Panax notoginseng* saponin extract.

Evaluation of Immunohistochemistry

Immunohistochemistry was conducted on liver tissue sections following a series of methodical procedures. Initially, after deparaffinization, the sections were incubated with 3% methanol-hydrogen peroxide for 10 minutes to quench endogenous peroxidase activity, followed by three washes with phosphate-buffered saline (PBS) for 5 minutes each. Subsequently, antigen retrieval was performed by treating the sections with citrate buffer (pH 6.0) and heating them to boiling in a microwave. The sections were then rinsed twice with PBS for 5 minutes each and subsequently blocked with goat serum for 20 minutes to prevent non-specific binding. Following overnight incubation at 4°C with rabbit anti-Nrf2 (1:200) and HO-1 (1:200), the sections were immersed in

phosphate-buffered saline (PBS, pH 7.4) and subjected to three consecutive washes of 5 minutes each using a decolourising shaker. Subsequently, the sections were gently dried by shaking and then treated with rabbit anti-HRP labeled antibody (1:2000) applied dropwise within a circumscribed area, followed by incubation at room temperature for 50 minutes. Specific labelling was achieved using a 3,3'-diaminobiphenyl diamine (DAB) kit, resulting in yellow or tan staining, while counterstaining of the nuclei was performed to assess the intensity of the staining and images were captured using a microscope. Immunohistochemical (IHC) staining was quantified as relative positive expression and any potential differences between groups were analysed using ImageJ software to identify areas of staining.

Ethical approval

This protocol was approved by the Animal Ethics Committee of Dali University (No. 2022-3011).

STATISTICAL ANALYSIS

Analyses of variance were performed using one-way analysis of variance (ANOVA) with SPSS version 27.0 (IBM). Statistical significance was defined as a P-value of less than 0.05, while P-values of less than 0.01 were considered highly significant.

RESULTS

High-performance liquid chromatography (HPLC) analysis was conducted to identify the chemical constituents of the saponin extract from *Panax notoginseng*. The analysis revealed the presence of twelve compounds, specifically *Panax notoginseng* saponin R1, and the ginsenosides Rk3, Rh4, Rg3, CK, Rg1, Rb1, Rc, Rb2, Rb3, Rd and F2. The concentrations of these compounds are detailed in table 2.

Effect of saponin extracts of Panax notoginseng on body weight

All groups of mice exhibited consistent growth throughout the duration of the experiment. As depicted in fig. 2A, during the eighth week, the body weight of mice administered low, medium and high doses of the extract was significantly lower compared to the model group ($P<0.05$).

The model group of mice exhibited a statistically significant increase in liver index compared to the normal group, as illustrated in fig. 2B. Notably, a significant reduction in liver index was observed in both the simvastatin group and the *Panax notoginseng* saponin extract group when compared to the model group ($P < 0.01$). The *Panax notoginseng* saponin extract demonstrated efficacy in reducing the liver index in hyperlipidemic mice.

Saponin extract of Panax notoginseng elevates hepatic SOD activity and reduces MDA content in hyperlipidemic mice

SOD activity and MDA levels serve as reliable biomarkers for hepatic oxidative stress and can be quantified in hyperlipidemic mice. In the model group, a significant reduction in SOD activity ($P<0.01$) and a significant increase in MDA content ($P<0.01$) were observed. Notably, the high-dose saponin extract group demonstrated a significant enhancement in SOD activity ($P<0.05$) compared to the model group. Additionally, both the simvastatin group and the saponin extract group exhibited significant reductions in MDA content ($P<0.05$). These findings suggest that *Panax notoginseng* saponin extracts possess potential efficacy in attenuating hepatic oxidative stress.

Panax notoginseng saponin extract can alter dyslipidemia in hyperlipidemic mice

At the fourth week, the seru TC levels in the simvastatin and *Panax notoginseng* saponins extract groups exhibited a significant increase compared to the model group ($P<0.05$). By the seventh week, simvastatin and medium to high doses of *Panax notoginseng* saponins extract significantly decreased serum TC levels relative to the model group ($P<0.01$ or $P<0.05$). By the eighth week, both simvastatin and *Panax notoginseng* saponins extract demonstrated a significant reduction in serum TC levels ($P<0.01$).

Throughout the study, dynamic fluctuations in serum TG levels were observed in mice. By the fourth week, a statistically significant elevation in serum TG levels was noted in the model group, the simvastatin group, and the groups receiving low, medium and high doses of *Panax notoginseng* saponins extract, in comparison to the normal group ($P<0.01$). Mice treated with *Panax notoginseng* saponins extract and simvastatin exhibited significantly lower serum triglyceride (TG) levels by the fifth week compared to the model group. Additionally, a significant reduction in serum TG levels was noted in the simvastatin group by the seventh week ($P<0.01$). By the eighth week, mice receiving both simvastatin and *Panax notoginseng* saponins extract demonstrated significant improvements in serum TG levels ($P<0.01$ or $P<0.05$).

A significant reduction in HDL-C levels was observed in the model group at the fourth week when compared to the normal group, particularly in relation to simvastatin and the low, medium, and high doses of *Panax notoginseng* saponin extract ($P<0.01$). Conversely, a notable increase in serum HDL-C levels was recorded in mice treated with simvastatin and the low, medium, and high doses of *Panax notoginseng* saponins at weeks five, six, and eight, in comparison to the model group. Notably, the high doses of *Panax notoginseng* saponins extract significantly ameliorated the condition ($P<0.01$ or $P<0.05$).

Table 1: Scoring criteria for liver pathology

Content of scoring	Score
Hepatocyte morphology is neat, hepatic cords are well arranged, Neither inflammatory cells nor hemorrhages are present	0 score
Disturbed arrangement of hepatic cords, occasional lipid droplet vacuoles, minor inflammatory cell infiltration or haemorrhage	1 score
Hepatocytes with mild swelling, more lipid droplet vacuoles, inflammatory cell infiltration or haemorrhage	2 score
Loss of hepatic sinusoids, heavy swelling of hepatocytes, numerous lipid droplet vacuoles or inflammatory infiltrates or marked haemorrhage	3 score
Large vacuolike lesions with inflammatory necrosis of hepatocytes	4 score

Table 2: Regression equations, linear correlation coefficients and contents of saponins

Retention time (MIN)	Sosaponin composition	Regression equation	Linearity (R^2)	Linearity range (μG)	Content (MG/G)
29.691	R1	y = 534724x + 4086.9	0.9997	0.4144-8.288	59.01
31.286	Rg1	y = 608590x - 1655.8	0.9996	0.3632-7.264	210.35
44.631	Rb1	y = 459612x + 262.57	0.9997	0.3504-7.008	31.68
46.1	Rc	y = 485828x - 2401.8	0.9995	0.2416-4.832	29.14
47.359	Rb2	y = 615684x + 28565	0.9997	0.500-10.000	12.13
48.002	Rb3	y = 390380x + 11508	0.9998	0.1728-3.456	65.92
50.449	Rd	y = 535885x - 4261.2	0.9997	0.4112-8.224	221.91
59.123	F2	y = 677525x - 3227	0.9997	0.1872-3.744	15.68
60.684	Rk3	y = 592374x - 59139	0.9997	0.084-2.520	43.25
61.662	Rh4	y = 365171x - 1690.1	0.9992	0.0576-1.152	60.58
64.46	Rg3	y = 477663x - 9288.4	0.9997	0.3186-6.336	8.83
74.718	CK	y = 866796x - 7524.6	0.9997	0.1440-2.880	14.79

At the fourth week, a significant increase in LDL-C levels was observed in the model group, simvastatin group and medium-dose *Panax notoginseng* saponins extract group ($P<0.01$ or $P<0.05$). However, by the fifth week, the simvastatin group and the groups receiving low and medium doses of *Panax notoginseng* saponins extract demonstrated a significant reduction in serum LDL-C levels ($P<0.01$). By the eighth week, mice treated with simvastatin and low and medium doses of *Panax notoginseng* saponins extract exhibited significant decreases in serum LDL-C levels ($P<0.05$).

Panax notoginseng saponin extract increased the apoA content in the serum of hyperlipidemia mice and decreased the apoB content

Apolipoprotein A (apoA) and Apolipoprotein B (apoB) are the primary lipoproteins present in the bloodstream, and they play crucial roles in lipid metabolism and cardiovascular health. In a comparative study involving different groups of mice, the model group exhibited a significant reduction in serum apoA levels and a significant increase in serum apoB levels compared to the normal group ($P<0.01$). Furthermore, at the fourth week of the experiment, a significant increase in serum apoA levels was observed in mice treated with simvastatin and *Panax notoginseng* saponin extract compared to the model group ($P<0.01$). Conversely, the serum apoA levels in mice treated with simvastatin, as well as medium and high doses of *Panax notoginseng* saponin extract, were significantly lower relative to the model group ($P<0.01$).

Panax notoginseng saponin extract relieved the liver pathological damage in hyperlipidemic mice

An elevation in the pathological score of liver tissue was observed in the model group relative to the control group ($P<0.01$). Conversely, the pathological scores of liver tissue in the simvastatin and high-dose *Panax notoginseng* saponins extract groups were significantly reduced compared to the model group ($P<0.05$), as illustrated in fig. 4C. Histological analysis demonstrated that the nuclei of blue-stained cells in the control group mice were distinctly visible, exhibiting minimal pathological changes in liver cells and occasional lipid droplets. Conversely, liver cells in the model group demonstrated disorganized cord arrangement, vacuolar-like lesions, a significant presence of lipid droplets, inflammatory cell infiltration or hemorrhaging, and a loss of liver sinusoids. In contrast, mice treated with low and medium doses of *Panax notoginseng* saponins extract exhibited minimal histopathological changes relative to the model group. The simvastatin-treated group showed reduced cellular swelling and a decrease in the number of inflammatory lesions in liver cells, although a notable presence of lipid droplets persisted. The high-dose group exhibited sporadic lipid droplets and a marked reduction in hepatic swelling and inflammatory lesions. These

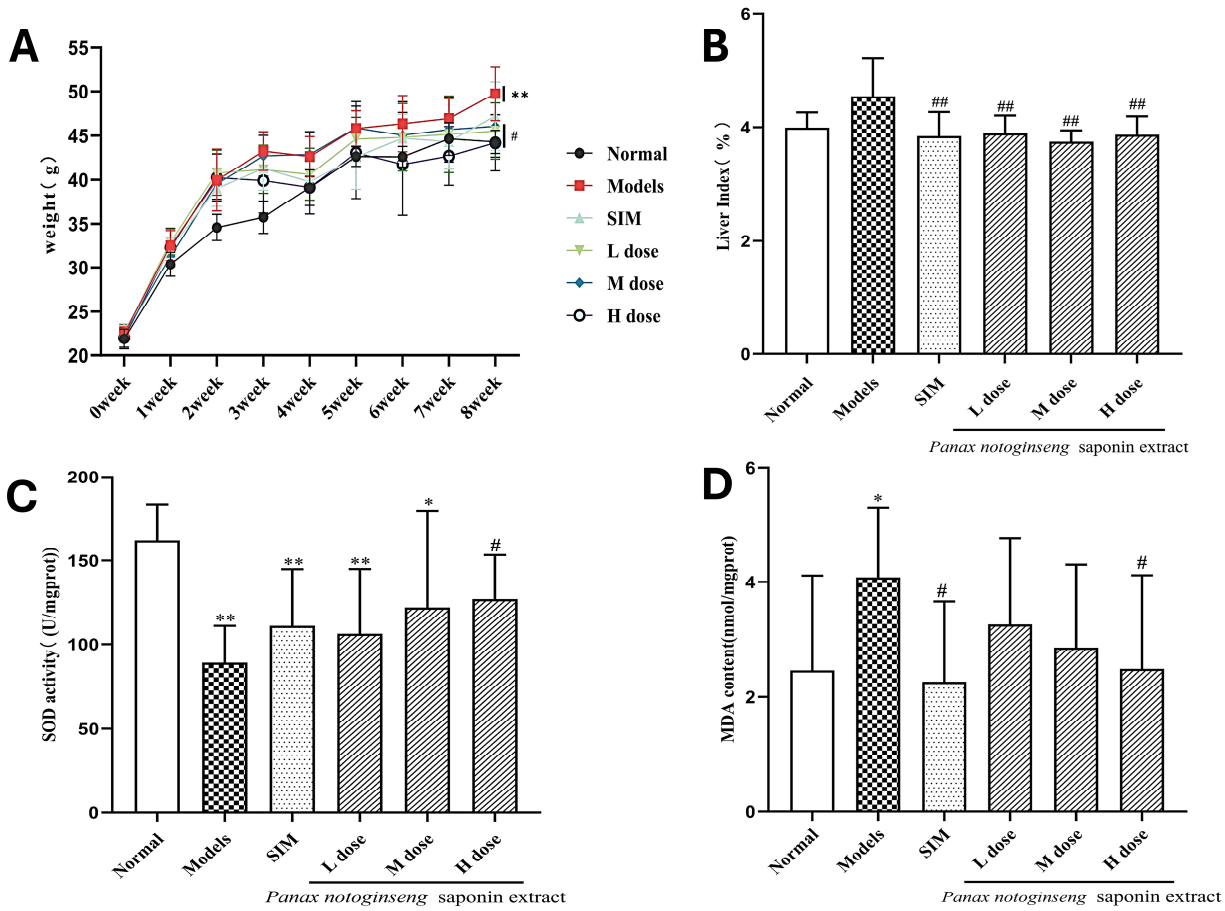

Fig. 2: Effects of *Panax notoginseng* saponin extract (high dose: 200 mg/kg; medium dose: 100 mg/kg low dose: 50 mg/kg) on body weight, liver index, apolipoprotein, Liver SOD Vitality and MDA contentin hyperlipidemic mice.(A) body weight, (B) liver index,(C) SOD Vitality (D) MDA contentin Effect of changes in data were presented as mean ± standard deviation (n = 8 per group).

*P<0.05, **P<0.01 Statistically significant between normal and other groups; $^{\#}P$<0.05, $^{\#\#}P$<0.01 Statistically significant between model and other groups.

observations suggest that initial exposure to mold induced mild hepatic damage, which was subsequently mitigated by pharmacological intervention. The results clearly demonstrate that saponin extracts of *Panax notoginseng* effectively ameliorate pathological liver injury in hyperlipidemic mice.

Saponin extract of Panax notoginseng alleviates hepatic lipid accumulation in hyperlipidaemic mice

Red Oil O staining was performed to evaluate lipid accumulation, with the findings illustrated in fig. 4C. Liver sections from the control group of mice demonstrated a minimal presence of lipid droplets. In contrast, the model group exhibited a substantial increase in lipid droplets, indicating successful induction of lipid accumulation via a high-fat diet. Notably, both the quantity and size of liver lipid droplets were significantly reduced when a high-dose *Panax notoginseng* saponins extract was administered in conjunction with simvastatin. These results suggest that the *Panax notoginseng* saponins extract effectively mitigates lipid accumulation.

Panax notoginseng saponin extract activates the Nrf 2 / HO-1 pathway

Fig. 5 depicts the expression of Nrf2 and HO-1 proteins via immunohistochemistry (ICH) to further investigate the impact of *Panax notoginseng* saponins extract on the Nrf2/HO-1 pathway. In the model group, the levels of Nrf2 and HO-1 proteins were markedly lower compared to the control group following administration of *Panax notoginseng* saponin extract. Conversely, the high-dose *Panax notoginseng* saponin extract group exhibited an elevation in Nrf2 and HO-1 protein levels, indicating a potential stimulatory effect on the Nrf2/HO-1 pathway *in vivo*.

DISCUSSION

Hyperlipidemia is acknowledged as a significant risk factor for both cardiovascular and cerebrovascular diseases, characterized by a complex pathogenesis. In the context of traditional Chinese medicine, the pathological manifestations of hyperlipidemia are linked to phlegm

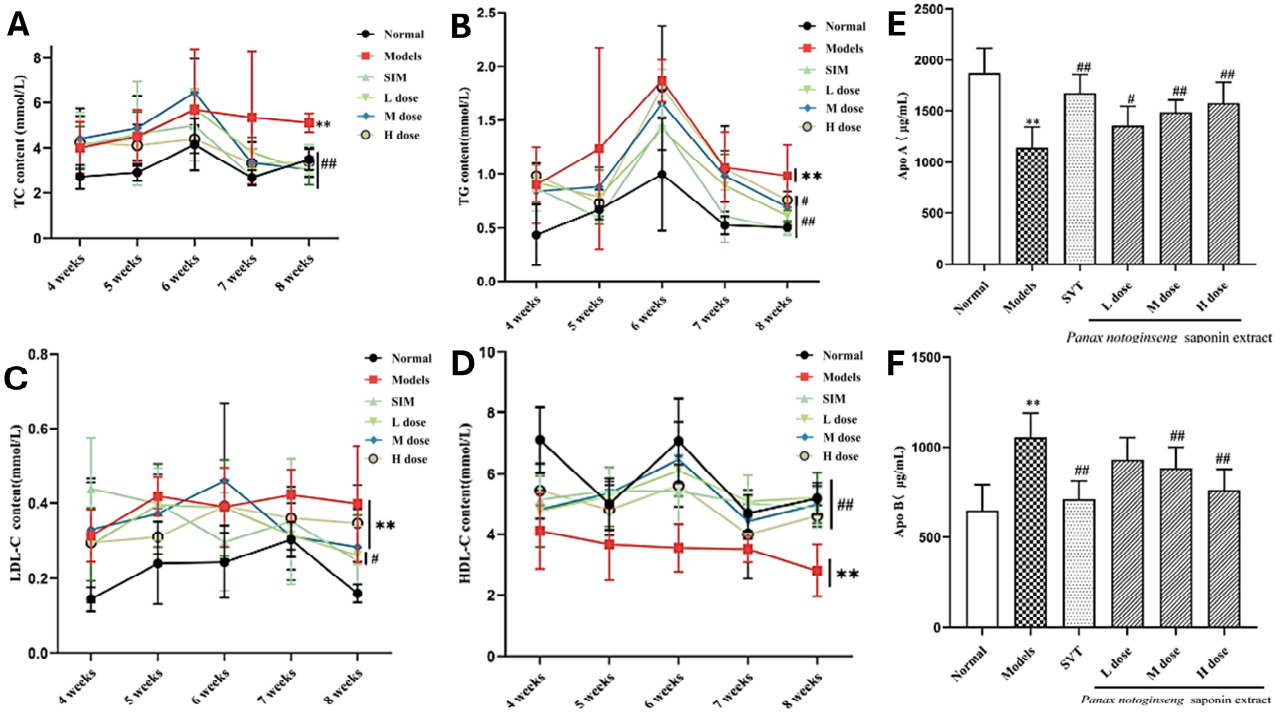

Fig. 3: Effects of Panax ginseng saponins (high dose: 200mg/kg; medium dose: 100mg/kg low dose: 50mg/kg) on apolipoprotein, TC, TG, LDL-C, HDL-C hyperlipidemic mice: (A) TC, (B) TG, (C) LDL- C, (D) HDL-C, (E) Apo A, (F) ApoB Effect of changes in . Data were presented as mean ± standard deviation (A-D:n = 6 per group, E-F: n = 8 per group).

*P<0.05, **P<0.01 Statistically significant between normal and other groups; $^{\#}P$<0.05, $^{\#\#}P$<0.01 Statistically significant between model and other groups.

dampness and blood stasis. *Panax notoginseng* is reputed to exhibit properties that enhance blood circulation, alleviate blood stasis, reduce inflammation and mitigate pain. However, the therapeutic properties of its extract, characterized by multiple compounds, targets and pathways, continue to obscure a comprehensive understanding of its mechanism in treating hyperlipidemia. This study demonstrates that *Panax notoginseng* saponin extract, when fermented by Cordyceps militaris, exhibits a significant therapeutic effect on hyperlipidemic mice. Through chemical characterization of the fermented *Panax notoginseng* saponin extract, we identified 9 rare saponins. In hyperlipidemic mice, elevated liver index, serum total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C) levels significantly impair health. However, administration of *Panax notoginseng* saponin extract mitigates these adverse effects.

ApoA (Qu *et al*., 2019) is the main lipoprotein carrier of HDL, which transports cholesterol to the liver for decomposition and metabolism, thereby reducing the accumulation of free cholesterol. Its content increases with the increase of HDL. ApoB (Morita S *et al*., 2016) is the main lipoprotein carrier of LDL, which transports cholesterol from the liver to liver tissue and decreases with the decrease of LDL. After treatment with *Panax notoginseng* saponins extract, the serum HDL-C level in mice increased and LDL-C level decreased. The content of ApoA increased and ApoB decreased, which is consistent with the above results. The liver mainly participates in glucose and lipid metabolism (Frazier *et al,* 2023). The findings from the histopathological examination revealed that the liver of the experimental group exhibited lesions, with disorganized arrangement of mouse liver cell cords and vacuolar degeneration observed in liver cells. Additionally, a significant presence of lipid droplets was noted within the liver cells and inflammatory cells infiltrated or bled. The sinusoids disappeared. In contrast, only a few lipid droplets were observed in the high-dose *Panax notoginseng* saponins extract group. The extent of swelling exhibited a notable decrease, the quantity of inflammatory lesions in liver cells was significantly diminished and the pathological score reached its minimum level. This indicates that *Panax notoginseng* saponins extract can alleviate fatty liver caused by hyperlipidemia to a certain extent, thereby protecting the liver and reducing the pathological score. The findings from the oil red O staining analysis indicated that the administration of *Panax notoginseng* saponins extract resulted in a decrease in hepatic lipid accumulation, demonstrating a potential protective impact.

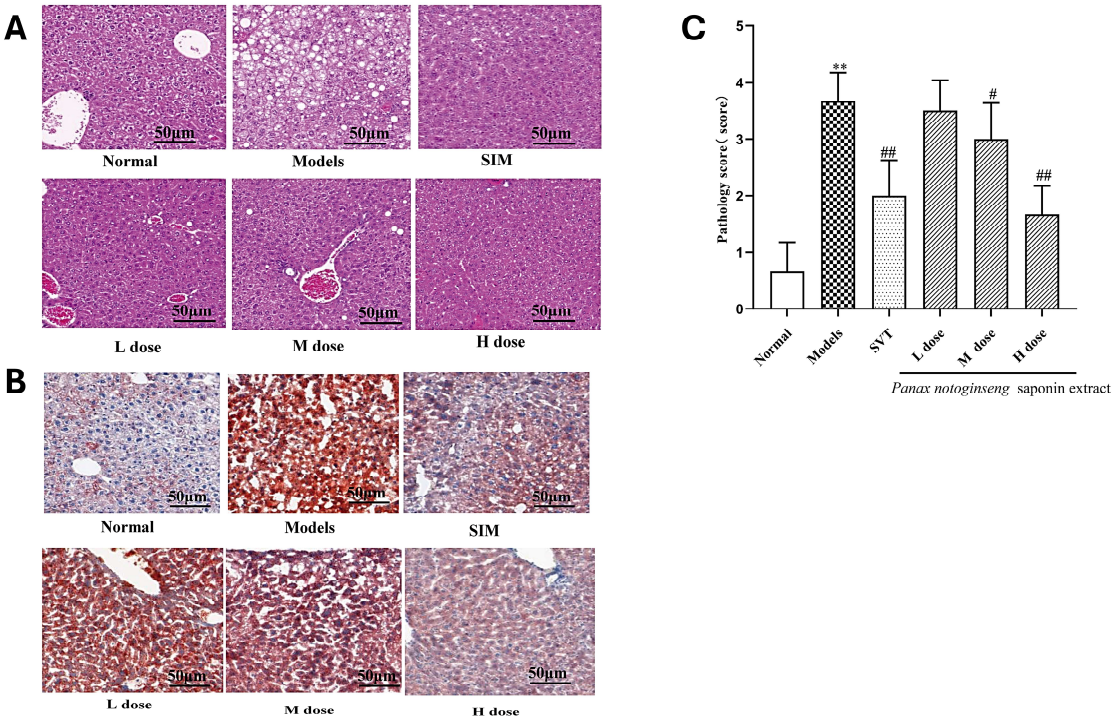

Fig. 4: Effect of *Panax notoginseng* saponin (high dose: 200 mg/kg; medium dose: 100 mg/kg low dose: 50 mg/kg) on liver histomorphology and liver histopathology score and Red oil 0 stain in hyperlipidemic mice: (A) changes in liver histomorphology, (B) Red oil 0 stain (C) liver histopathology score(s (H&E, Red oil 0 stain, × 200). Data were presented as mean ± standard deviation (n = 8 per group).
*P<0.05, **P<0.01 Statistically significant between normal and other groups; $^{\#}P$<0.05, $^{\#\#}P$<0.01 Statistically significant between model and other groups.

The development of hyperlipidemia involves a multifaceted process that is primarily characterized by its complexity, includes endoplasmic reticulum stress (Li *et al*., 2018), gene polymorphism, inflammatory factors (Guo *et al*., 2019), oxidative stress (Cheng *et al*., 2024), and intestinal flora (Agus *et al*., 2021). Under physiological conditions, the oxidation and antioxidation of the human body are in a balanced state. When it is in a pathological state for a long time such as high-fat state, the content The elevation of reactive oxygen species (ROS) levels within the organism is heightened. leading to an imbalance between oxidation and antioxidation. The oxidation reaction is further strengthened, causing damage to tissues and cells (Bai *et al*., 2021). In the high-fat state, the body's oxidation reaction is higher than that of normal people, and the antioxidant capacity is reduced. This leads to a decrease in antioxidant enzyme levels, promotes lipid oxidation, and causes lipid accumulation in the body (Ta *et al*., 2023), which can cause diseases such as atherosclerosis and increase, the likelihood of developing cardiovascular disease. When the lipid in the body is too high or excessive ROS is produced, superoxide dismutase (SOD) can protect plasma lipids from oxidation (Bai *et al*., 2021). In the body, SOD effectively eliminates O2- by causing it to undergo disproportionation to form H2O2 and O2, reducing the damage free radicals can cause. Malondialdehyde (MDA) is an indicator of lipid peroxidation in the cell membrane. *Panax notoginseng* saponins extract mildly ameliorated these effects, suggestive of lipid peroxidation and cellular damage in the experimental mice.

The nuclear factor erythroid 2-related factor 2 (Nrf2) activates genes that protect against antioxidant and electrophile stress. This enzyme regulates downstream genes involved in antioxidant stress, such as heme oxygenase-1 (HO-1) and superoxide dismutase (SOD).This factor is essential in governing cellular defense mechanisms against stress conditions. It controls the activity of antioxidant enzymes by attaching to antioxidant response elements (AREs) (Li *et al*., 2023, Qiong-Lian Fang *et al*., 2023). Hepatic fatty acid metabolism is also regulated by Nrf2. Increasing evidence suggests that Nrf2 exerts a cellular protective influence (Cuadrado *et al*., 2019, Bollong, *et al*., 2018). The transcription factor Nrf2 activates HO-1 under oxidative stress (Shen *et al*., 2019). HO-1 can decompose heme into CO and bilirubin, providing protective functions In the cardiovascular system, the impacts of carbon monoxide (CO) and bilirubin on different cell types are observed. (Yamamoto *et al*., 2018; Facchinetti *et al*., 2020; Abd El-Twab *et al*., 2019) A variety of benefits have been demonstrated for the enzyme heme oxygenase-1 (HO-1) including anti-apoptotic, anti-inflammatory, anti-

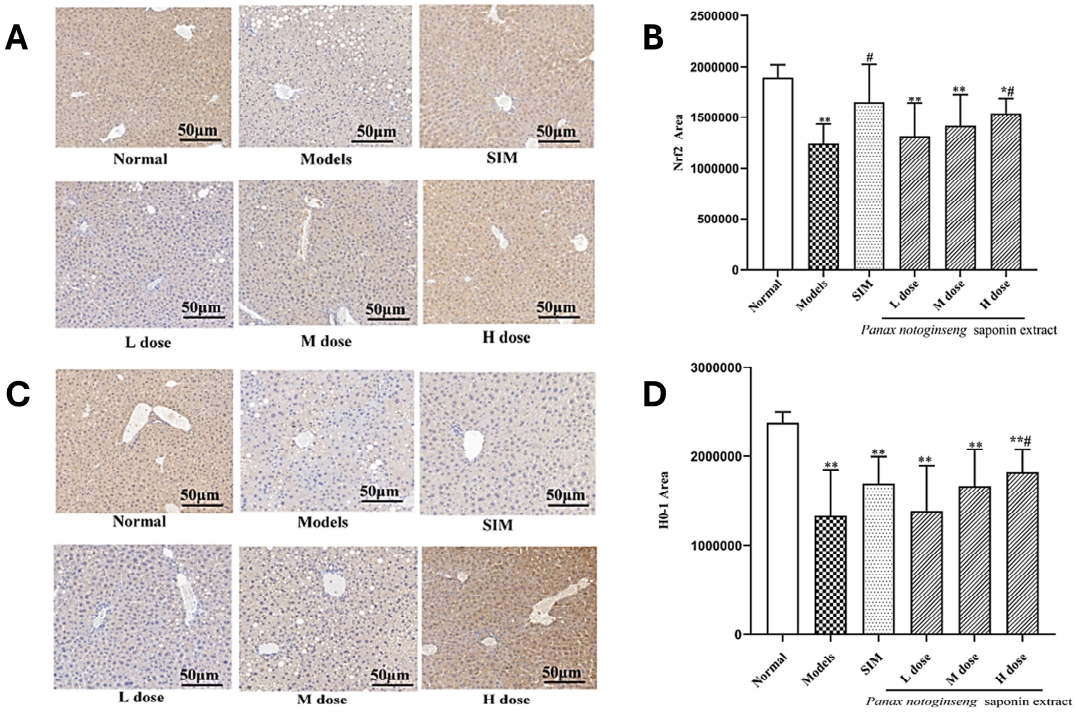

Fig. 5: Effect of *Panax notoginseng* saponin (high dose: 200 mg/kg; medium dose: 100 mg/kg low dose: 50mg/kg) on hepatic HO-1,Nrf2 protein expression in hyperlipidemic mice: (A) hepatic HO-1 protein expression, (B) effect of protein Nrf2 protein expression (×200). Data were presented as mean ± standard deviation (n = 8 per group).
**P*<0.05, ***P*<0.01 Statistically significant between normal and other groups; #*P*<0.05, ##*P*<0.01 Statistically significant between model and other groups.

hypertensive and antioxidant properties. The researchers examined liver Nrf2 and HO-1 levels using immunohistochemistry (ICH). *Panax notoginseng* saponins extract increased Nrf2 and HO-1 protein expression. By activating the Nrf2/HO-1 pathway, *Panax notoginseng* saponins may reduce blood lipid levels and oxidative stress.

CONCLUSION

Panax notoginseng saponin extract has a therapeutic effect on hyperlipidemia in mice. There may be an association between this phenomenon and the stimulation of the Nrf2/HO-1 signaling pathway. Enhancement of the oxidative stress response, relief of liver pathological damage, reduction of liver lipid accumulation, and regulation of blood lipid levels in hyperlipidemic mice.

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