

# Intervention Effects of Atorvastatin Combined with *Panax notoginseng* Saponins on Rats with Atherosclerosis Complicated with Hepatic Injury

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## ABSTRACT

**Background:** Statins cannot be used for some active liver diseases, which limits its application to some extent. The combined use of statins with other drugs may be one of the ways to solve this dilemma. **Objective:** This research aims to evaluate the effects of atorvastatin combined with *Panax notoginseng* saponins (PNS) on rats with atherosclerosis (AS) complicated with hepatic injury. **Materials and Methods:** Seventy-two male Wistar rats were randomly categorized into control group (without any intervention, Group A) and AS model groups, which were divided into hepatic injury (Groups B–E) and nonhepatic injury (Groups F–I) groups. Hepatic and nonhepatic injury groups were intragastrically treated with 5.5 mg/kg-d atorvastatin (Group B, F), 200 mg/kg-d PNS (Group C, G), 5.5 mg/kg-d atorvastatin + 200 mg/kg-d PNS (Group D, H), and normal saline (Group E, I). After 8 weeks, total cholesterol (TC), triglyceride (TG), high density lipoprotein-cholesterol, low density lipoprotein-cholesterol (LDL-C), and serum calcium were analyzed to evaluate the hypolipidemic effect. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, total bilirubin, and  $\gamma$ -glutamyltransferase levels were measured to assess liver function. The thoracic aortas were used for hematoxylin–eosin staining. **Results:** In both hepatic injury and nonhepatic injury groups, TC, TG and LDL-C levels significantly decreased in Groups B, D, F, and H. ALT and AST levels significantly increased in Group B, but significantly decreased in Groups C and D. The aortic intima thickness was significantly lower in Groups B, D, F, and H than that in the normal saline group. **Conclusion:** The combination of atorvastatin and PNS treatment showed a significant hypolipidemic effect and hepatic enzyme stability function.

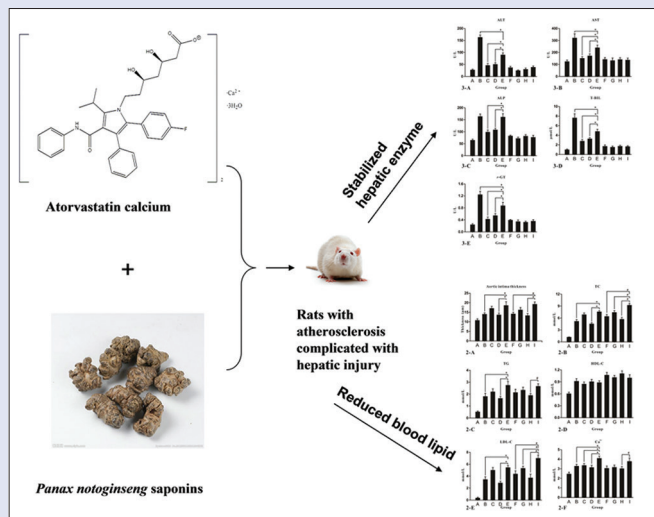
**Key words:** Atherosclerosis, atorvastatin, hepatic injury, *Panax notoginseng*

## SUMMARY

- The single use of *Panax notoginseng* saponins (PNS) in the rat model for atherosclerosis significantly reduced  $Ca^{2+}$  content in serum, whereas the effect of lowering total cholesterol (TC), triglyceride (TG), and low density lipoprotein-cholesterol (LDL-C) is not apparent, especially as compared with atorvastatin treatment
- PNS combined with atorvastatin treatment of the rat model for atherosclerosis displayed a noticeable, synergistic effect that allowed for better reduction of TC, TG, LDL-C and  $Ca^{2+}$  in the serum than that with the single use of PNS or atorvastatin
- In the rat liver injury combined with atherosclerosis model, the single use of PNS

significantly improved liver function, whereas atorvastatin alone only aggravated liver injury in the rat model. The effect of PNS combined with atorvastatin on liver function was significantly better than that of atorvastatin alone

- The combined use of PNS and atorvastatin showed good stability of liver function on the liver injury combined with atherosclerosis model.



**Abbreviations used:** PNS: *Panax notoginseng* saponins; AS: Atherosclerosis; TC: Total cholesterol; TG: Triglyceride; HDL-C: High density lipoprotein-cholesterol; LDL-C: Low density lipoprotein-cholesterol; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; T-BIL: Total bilirubin; r-GT:  $\gamma$ -glutamyltransferase; HE: Hematoxylin–eosin.

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## INTRODUCTION

Cardiovascular diseases have been the primary cause of death and have contributed to increased total death rate worldwide in the past 100 years.<sup>[1]</sup> Atherosclerosis (AS) is the main cause of death among cardiovascular diseases.<sup>[2]</sup> AS is characterized by chronic degeneration of arteries and gradual changes in the artery wall. Inflammation is considered to be a necessary condition in the induction of AS.<sup>[3]</sup>

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The artery wall hardens and thickens, and the whole artery loses flexibility because of the growth of connective tissues; deposition of cholesterol, fatty acids, intracellular and extracellular calcium carbonate; and aggregation of collagen and protein.<sup>[2]</sup> Cholesterol and other substances accumulate to a specific degree, and endothelial cells of the vascular wall induce the differentiation of monocytes into macrophages.<sup>[4]</sup> Macrophages phagocytize fats between the artery walls, allowing them to accumulate in the cell and converting endothelial cells into foam cells. Dyslipidemia is an important factor that contributes to the pathogenesis of atherosclerotic disease.<sup>[5,6]</sup> A decrease in total cholesterol (TC) of 1 mg/dL can decrease the atherosclerotic disease incidence by 2%.<sup>[7]</sup> A large number of randomized controlled clinical studies<sup>[8]</sup> also confirm the effectiveness of statins as first-line medicine in the lipid-lowering treatment of cardiovascular diseases. Apart from having lipid-lowering effect, attenuation of inflammation, plaque stabilization and improvement of endothelial dysfunction may contribute to potential benefits of statins therapy.<sup>[9]</sup> Over the past 10 years, a large number of clinical studies have evaluated the safety and efficacy of the long-term use of statins. However, the instructions of Lipitor tablets state that atorvastatin cannot be used for active liver diseases, including elevated liver aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) levels for unknown reasons, thereby limiting the application of this medication to some extent.

Statins may lead to extensive hepatic adverse reactions. In the early stage of treatment with statins, temporary increase in transaminase levels do not manifest into clinical symptoms.<sup>[10,11]</sup> This phenomenon could be due to changes in liver cellular membrane through enzyme leakage rather than by direct liver injury.<sup>[12]</sup> Changes in the lipid component of the modified liver cellular membrane may result in increased permeability and leakage of liver enzyme.<sup>[13,14]</sup> This phenomenon is characteristic of all lipid-lowering agents and may be secondary to the lipid-lowering process itself, such that the effect is not exclusive to statin drugs.<sup>[15]</sup>

Medicinal plants are significant sources of hepatoprotective drugs.<sup>[16]</sup> *Panax notoginseng* is used in traditional Chinese medicine as a valuable medicinal herb. *P. notoginseng* saponins (PNS) are the active components<sup>[17,18]</sup> of *P. notoginseng*. Although several studies have shown<sup>[19,20]</sup> that PNS positively influences blood lipid regulation, antioxidation, anti-inflammation, and angiogenesis, the anti-atherosclerotic mechanism of PNS remains unclear. PNS can prevent lipid deposition in blood vessels, inhibit the proliferation of vascular smooth muscle cells, scavenge free radicals, inhibit platelet aggregation, and promote blood circulation.<sup>[21,22]</sup> A systematic review indicated that PNS can prevent stroke.<sup>[23]</sup>

PNS can resist liver injury.<sup>[24]</sup> The effective rate of PNS injection treatment in 58 cases of refractory blood stasis type of chronic hepatitis reached 80%.<sup>[25]</sup> Daily oral treatment of *P. notoginseng* powder at 4.5–6.0 g for chronic persistent hepatitis in 49 cases exhibited a total effective percentage as high as 93.8%. The hepatic protective effect of PNS is mainly manifested in the reduction of hepatic cell degeneration and necrosis as well as decrease in the amount of collagen fibers among hepatic cells. PNS also elicits a certain cholagogic effect,<sup>[26]</sup> which can significantly reduce serum bilirubin and promote bile secretion.

This study established a rat AS model by using a large dose of Vitamin D<sub>3</sub> as calcium ion inducer and administering high-fat fodder. Acute hepatic injury was induced by acetaminophen. Changes in TC, triglyceride (TG), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), and serum calcium (Ca<sup>2+</sup>) were examined after PNS and atorvastatin treatment. In addition, histopathological changes in the aorta were observed by hematoxylin–eosin (HE) staining. Liver function indicators such as ALT, AST, alkaline phosphatase (ALP), total bilirubin (T-BIL), and *r*-glutamyltransferase (*r*-GT) were examined.

## MATERIALS AND METHODS

### Experimental animals

In this experiment we followed the Guidelines for Care and Use of Laboratory Animals as approved by Guangzhou Medical University. Male Wistar rats (6 weeks old, weighed 150 ± 10 g on average) were purchased from the Experimental Animal Center of the Guangzhou University of Chinese Medicine (Guangzhou, China). The rats were fed in a controlled environment with a room temperature of 25°C ± 2°C and a relative humidity of 60% ± 15%, respectively, and a 12-h light–dark cycle. Experimental animals were allowed to adapt to the environment for 1 week, water and solid feed (Beijing Keao Xieli Feed Co., Ltd., Beijing) were unlimitedly supplied to the rats during the experiment.

### Test drugs and reagents

The materials used were as follows: vitamin D<sub>3</sub> (injection, purchased from Shanghai GM Pharmaceutical Co. Ltd., Shanghai, China), atorvastatin (atorvastatin calcium tablets, Lipitor, purchased from Pfizer Pharmaceuticals Co. Ltd., America), PNS (Xuesaitong injection (lyophilized), purchased from Haerbin Zhenbao Pharmaceutical Co., Ltd., Haerbin, China), acetaminophen (Sigma Chemical, America), pentobarbital sodium (Sigma Chemical, America), and reagent test kits (Ningbo Medical System Biotechnology Co., Ltd, Ningbo, China).

PNS in this study contained notoginsenoside R1 11.7%, ginsenoside Rg1 38.8%, ginsenoside Re 4.1%, ginsenoside Rb1 33.1% and ginsenoside Rd 4.6%. The chemical purity of PNS was 92.3%.

### Experimental atherogenic diet

The experimental atherogenic diet was prepared as follows: 80.3% normal basal rat diet, 10% lard, 5% yolk powder, 1% sugar, 3% cholesterol, 0.5% bile salts, and 0.2% propylthiouracil.

Normal basal rat diet consisted of corn, soybean meal, fish meal, wheat bran, flour, salt, calcium powder, vitamins, trace elements, and amino acid (Beijing Keao Xieli Feed Co., Ltd., Beijing).

### Experimental methods

#### Animal grouping

Seventy-two male Wistar rats were allowed to adapt to the environment for 1 week and randomly divided into nine groups: One blank control group without any intervention (Group A, *n* = 8), and eight model groups treated with high-fat fodder and intraperitoneal injection of Vitamin D<sub>3</sub> to establish the rat AS model. The model groups were divided into eight groups as follows: Groups B, C, D, and E were assigned as the liver injury groups and were intraperitoneally injected with 400 mg/kg acetaminophen at the fourth weekend to induce liver injury. Groups F, G, H, and I were assigned as the nonliver injury groups and were intraperitoneally injected with normal saline instead. The liver injury and nonliver injury groups were treated intragastrically with atorvastatin 5.5 mg/kg-d (Group B, *n* = 8; Group F, *n* = 8), PNS 200 mg/kg-d<sup>[27-29]</sup> (Group C, *n* = 8; Group G, *n* = 8), atorvastatin 5.5 mg/kg-d + PNS 200 mg/kg-d (Group D, *n* = 8; Group H, *n* = 8), and normal saline (Group E, *n* = 8; Group I, *n* = 8). The experimental rats received test drug intervention for 8 weeks.

### Experimental process

Group A was given normal basal rat diet. Groups B–I received daily intraperitoneal injection of Vitamin D<sub>3</sub> (0.25 million U/kg-d) for 3 days and were fed experimental atherogenic diet for 4 weeks to establish a rat AS model. At the fourth weekend, Groups B–E received 400 mg/kg intraperitoneal injection of acetaminophen to induce acute liver injury.

At the end of the 4<sup>th</sup> week, two rats were randomly selected in each of Groups A, E, and I to confirm the establishment of an AS model and a liver injury model. The rats were fasted for 12 h, weighed, and anesthetized. Blood was obtained from the right atrium of the rats after opening the chest. Serum was separated and measured for blood lipid (TC, TG, LDL-C, HDL-C, Ca<sup>2+</sup>) and liver function (ALT, AST, ALP, *r*-GT, T-BIL) indices by using an automatic biochemical analyzer. About 1 cm thoracic aorta was isolated, fixed, paraffin-embedded, and HE-stained. Histopathological changes in the aorta were observed under an optical microscope to determine whether an AS model was established.

At the 5<sup>th</sup> week, Groups B and F were given 5.5 mg/kg·d atorvastatin intragastrically, Groups C and G were given 200 mg/kg·d PNS, Groups D and H were given 5.5 mg/kg·d atorvastatin + 200 mg/kg·d PNS, and Groups E and I were given normal saline as control. The experimental rats received drug intervention test for 8 weeks. Throughout the entire course of the drug intervention, Group A was given normal saline as control.

## Measurement of blood lipid concentration and liver function

At the end of the 12<sup>th</sup> week of the experiment, all rats were fasted for 12 h, weighed, anesthetized with 2% pentobarbital sodium (0.3 mL/100 g) intraperitoneal injection. Blood samples (3 mL) were extracted from the right atrium after opening the chest, kept at room temperature for 30 min, then centrifuged at a speed of 2500 revolutions per minute for 15 min. Briefly, 1 mL of the upper serum was isolated to analyze blood lipid content and liver function. TC, TG, HDL-C, LDL-C, and serum Ca<sup>2+</sup> were analyzed using the plasma lipid profile. Serum ALT, AST, ALP, T-BIL and *r*-GT levels were measured to assess liver function.

### Total cholesterol

A mixture of 3 µL of the serum and 0.2 mL of enzyme reagent-1 was heated in a test tube at 37°C for 4 min. Optical absorbance was determined at 546 nm wavelength as A<sub>0</sub>. The mixture was added with 0.1 mL of enzyme reagent-2 and heated at 37°C for 5 min. Optical absorbance was determined at 546 nm wavelength as A<sub>1</sub>. ΔA was calculated using the formula: ΔA = A<sub>1</sub> - A<sub>0</sub>. Intra-serum TC level was calculated by the following formula (mmol/L):

$$\text{Concentration (TC)} = (\Delta A_{\text{Detection}} - \Delta A_{\text{Blank}}) / (\Delta A_{\text{Calibration}} - \Delta A_{\text{Blank}}) \times \text{concentration (calibration)}.$$

### Triglyceride

A mixture of 3 µL of the serum and 0.2 mL enzyme reagent-1 was heated in a test tube at 37°C for 4 min. Optical absorbance was determined at 546 nm wavelength as A<sub>0</sub>. The mixture was added with 0.1 mL enzyme reagent-2 and heated at 37°C for 5 min. Optical absorbance was determined at 546 nm wavelength as A<sub>1</sub>. ΔA was calculated using the formula: ΔA = A<sub>1</sub> - A<sub>0</sub>. Intra-serum TG level was calculated by the following formula (mmol/L):

$$\text{Concentration (TG)} = (\Delta A_{\text{Detection}} - \Delta A_{\text{Blank}}) / (\Delta A_{\text{Calibration}} - \Delta A_{\text{Blank}}) \times \text{concentration (calibration)}.$$

### High-density lipoprotein-cholesterol

A mixture of 3 µL of the serum and 0.3 mL of enzyme reagent-1 was heated in a test tube at 37°C for 5 min. Optical absorbance was determined at 600 nm wavelength as A<sub>0</sub>. The mixture was added with 0.1 mL of enzyme reagent-2 and heated at 37°C for 5 min. Optical absorbance was determined at 600 nm wavelength as A<sub>1</sub>. ΔA was calculated using the formula: ΔA = A<sub>1</sub> - A<sub>0</sub>. Intra-serum HDL-C level was calculated by the following formula (mmol/L):

$$\text{Concentration (HDL-C)} = (\Delta A_{\text{Detection}} - \Delta A_{\text{Blank}}) / (\Delta A_{\text{Calibration}} - \Delta A_{\text{Blank}}) \times \text{concentration (calibration)}.$$

### Low-density lipoprotein-cholesterol

Intra-serum LDL-C level was calculated using TC, TG, and HDL-C with the following formula (mmol/L):

$$\text{LDL-C} = \text{TC} - (\text{HDL-C} + \text{TG}/2.2)$$

### Ca<sup>2+</sup>

A mixture of 3 µL of the serum and 0.3 mL of the reagent was heated in a test tube at 37°C for 5 min. Optical absorbance was determined at 650 nm wavelength as A. Intra-serum Ca<sup>2+</sup> level was calculated by the following formula (mmol/L):

$$\text{Concentration (Ca}^{2+}\text{)} = (A_{\text{Detection}} - A_{\text{Blank}}) / (A_{\text{Calibration}} - A_{\text{Blank}}) \times \text{concentration (calibration)}.$$

### Alanine aminotransferase

A mixture of 15 µL of the serum and 0.2 mL of reagent-1 was heated in a test tube at 37°C for 3 min. The mixture was added with 0.1 mL of reagent-2 and heated at 37°C for 60 s. Optical absorbance was determined at 340 nm wavelength for 1–3 min. ΔA/min was then derived. Intra-serum ALT level was calculated by the following formula (U/L):

$$\text{Activity (ALT)} = (\Delta A_{\text{Detection}}/\text{min} - \Delta A_{\text{Blank}}/\text{min}) / (\Delta A_{\text{Calibration}}/\text{min} - \Delta A_{\text{Blank}}/\text{min}) \times \text{activity (calibration)}.$$

### Aspartate aminotransferase

A mixture of 15 µL of the serum and 0.2 mL of reagent-1 was heated in a test tube at 37°C for 3 min. The mixture was added with 0.1 mL of reagent-2 and heated at 37°C for 90 s. Optical absorbance was determined at 340 nm wavelength for 1–3 min. ΔA/min was then derived. Intra-serum AST level was calculated by the following formula (U/L):

$$\text{Activity (AST)} = (\Delta A_{\text{Detection}}/\text{min} - \Delta A_{\text{Blank}}/\text{min}) / (\Delta A_{\text{Calibration}}/\text{min} - \Delta A_{\text{Blank}}/\text{min}) \times \text{activity (calibration)}.$$

### Alkaline phosphatase

A mixture of 6 µL of the serum and 0.24 mL of reagent-1 was heated in a test tube at 37°C for 3 min. The mixture was added with 0.06 mL of reagent-2 and heated at 37°C for 60 s. Optical absorbance was determined at 405 nm wavelength for 1–3 min. ΔA/min was then derived. Intra-serum ALP level was calculated by the following formula (U/L):

$$\text{Activity (ALP)} = (\Delta A_{\text{Detection}}/\text{min} - \Delta A_{\text{Blank}}/\text{min}) / (\Delta A_{\text{Calibration}}/\text{min} - \Delta A_{\text{Blank}}/\text{min}) \times \text{activity (calibration)}.$$

### Total bilirubin

A mixture of 8 µL of the serum and 0.24 mL of reagent-1 was heated in a test tube at 37°C for 4 min. Optical absorbance was determined at 450 nm wavelength as A<sub>0</sub>. The mixture was added with 0.06 mL of reagent-2 and heated at 37°C for 5 min. Optical absorbance was determined at 450 nm wavelength as A<sub>1</sub>. ΔA was then calculated using the formula: ΔA = A<sub>1</sub> - A<sub>0</sub>. Intra-serum T-BIL level was calculated by the following formula (µmol/L):

$$\text{Concentration (T-BIL)} = (\Delta A_{\text{Detection}} - \Delta A_{\text{Blank}}) / (\Delta A_{\text{Calibration}} - \Delta A_{\text{Blank}}) \times \text{concentration (calibration)}.$$

### *r*-glutamyltransferase

A mixture of 15 µL of the serum and 0.24 mL of reagent-1 was heated in a test tube at 37°C for 4 min. The mixture was added with 0.06 mL of reagent-2 and heated at 37°C for 60 s. Optical absorbance was determined at 405 nm wavelength for 1–3 min. ΔA/min was then derived. Intra-serum *r*-GT level was calculated by the following formula (U/L):

$$\text{Activity (*r*-GT)} = (\Delta A_{\text{Detection}}/\text{min} - \Delta A_{\text{Blank}}/\text{min}) / (\Delta A_{\text{Calibration}}/\text{min} - \Delta A_{\text{Blank}}/\text{min}) \times \text{activity (calibration)}.$$

### Hematoxylin–eosin staining

At the end of the 12<sup>th</sup> week, after blood extraction, about 1 cm of thoracic aorta was isolated near the aortic arch, placed in 4% formaldehyde solution, and fixed for 24 h. The fixed blood vessel was dehydrated with gradient alcohol, made transparent with xylene solution, paraffin-embedded, and sectioned. The section was then dewaxed with xylene, sequentially exposed to high-to-low concentration of alcohol, flushed with distilled water, HE stained, dehydrated, made transparent, and fixed. The degree of smoothness and thickness of the aortic wall were observed under a light microscope. The arrangement of endometrial cells was examined to determine the presence of fat cells and foam cells. Five horizons were randomly selected and measured for aortic intimal thickness through a simple PCI image analysis software, then the values were averaged.

### Statistical analysis

SPSS 17.0 software (International Business Machines Corporation, America) was used for statistical processing, and (mean  $\pm$  standard error of the mean) was used to express the measurement data. Diversity of the average number of comparisons was assessed using one-way analysis of variance, where  $P < 0.05$  for the difference was statistically significant.

## RESULTS

### Histopathological observation of rat aortas in each group (hematoxylin–eosin staining)

In Group A, the vascular intima was smooth, thin and complete, and the cells were arranged neatly [Figure 1a]. In Group E, vascular proliferation was evident, the endothelial cells were disorderly arranged, and multiple irregular stripes were visible [Figure 1e]. These results confirm that the AS model was established successfully. Based on the sections, vascular endothelial hyperplasia in the medication-administered groups [Figure 1b-d and f-h] were improved by varying degrees compared with that in the saline group [Figure 1e and i].

### Aortic intima thickness and blood lipid of rats in each group

Aortic intima thickness and blood lipid indices of rats in each group were showed in Table 1 and Figure 2.

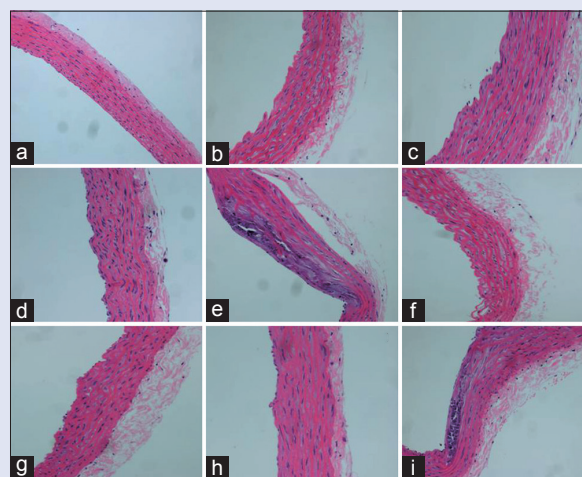
#### Aortic intimal thickness

In the liver injury groups, aortic intimal thickness of the atorvastatin (Group B) and atorvastatin combined with PNS (Group D) groups was significantly smaller than that of the saline group (Group E) ( $P < 0.05$ ), whereas the PNS (Group C) and saline (Group E) groups had no significant difference. In the nonliver injury groups, aortic intimal

thickness in the atorvastatin (Group F) and atorvastatin combined with PNS (Group H) groups was significantly smaller than that in the saline group (Group I) ( $P < 0.05$ ); conversely, the intimal thickness was not significantly different between PNS (Group G) and saline (Group I) groups.

#### Total cholesterol

In the liver injury groups, TC level in the atorvastatin (Group B) and atorvastatin combined with PNS (Group D) groups was significantly lower than that in the saline group (Group E) ( $P < 0.05$ ). TC level was not significantly different between PNS (Group C) and saline (Group E) groups. In the nonliver injury groups, TC level in the atorvastatin (Group F), PNS (Group G), and atorvastatin combined with PNS (Group H)

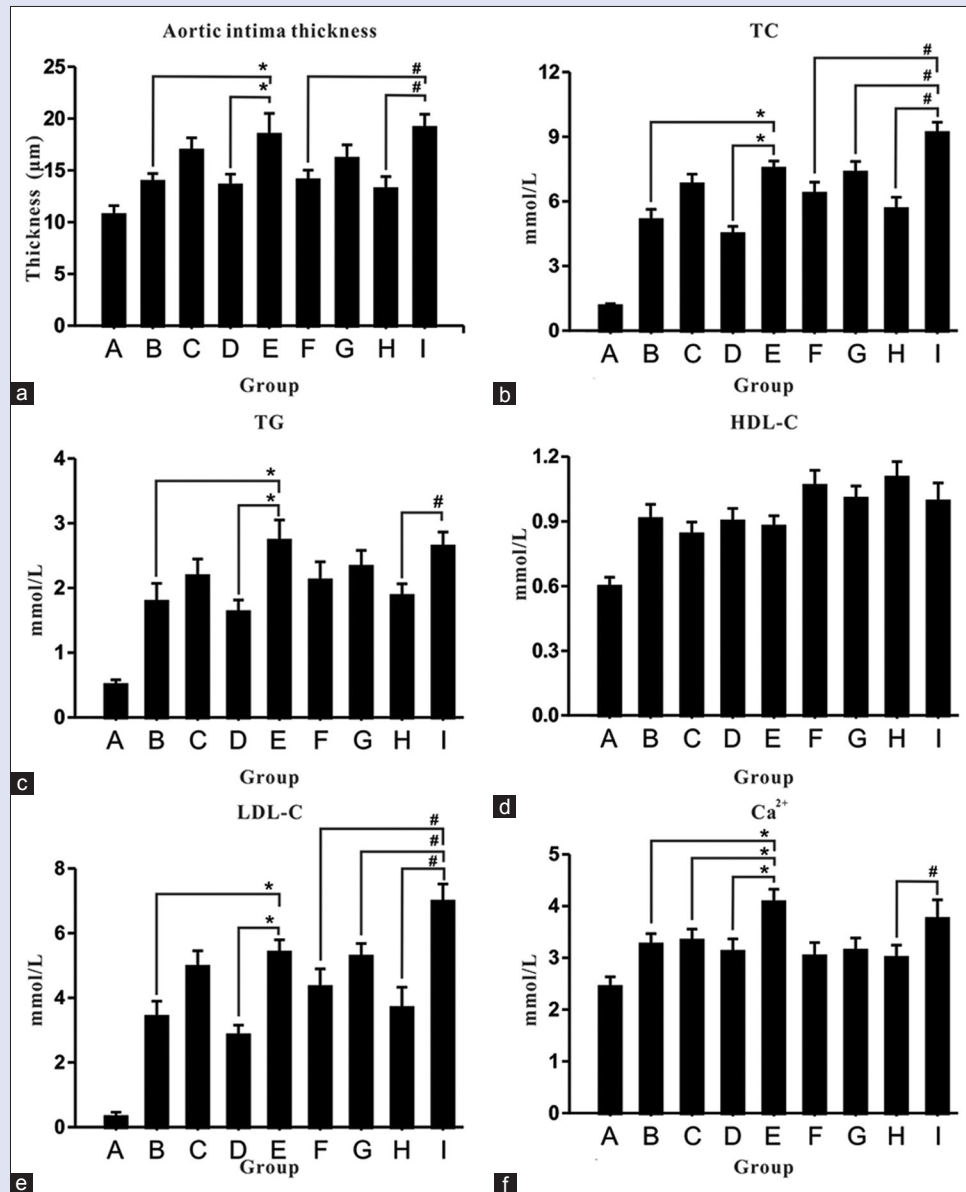


**Figure 1:** Histopathological observation of atherosclerotic pathology alteration in rat aortas (hematoxylin–eosin staining). (a) Normal control group, rats were fed a normal diet and received no other treatment. (b) Atorvastatin intervention group, rats were induced to liver injury complicated with atherosclerosis; (c) Panax notoginseng saponins intervention group, rats were induced to liver injury complicated with atherosclerosis; (d) atorvastatin combined with Panax notoginseng saponins intervention group, rats were induced to liver injury complicated with atherosclerosis; (e) normal saline intervention group, rats were induced to liver injury complicated with atherosclerosis; (f) atorvastatin intervention group, rats were induced to atherosclerosis with no liver injury; (g) Panax notoginseng saponins intervention group, rats were induced to atherosclerosis with no liver injury; (h) atorvastatin combined with Panax notoginseng saponins intervention group, rats were induced to atherosclerosis with no liver injury; (i) normal saline intervention group, rats were induced to atherosclerosis with no liver injury

**Table 1:** Aortic intima thickness and blood lipid of rats in each group

Group	Aortic intima thickness ( $\mu\text{m}$ )	Blood lipid (mmol/L)				
		TC	TG	HDL	LDL-cholesterol	Ca <sup>2+</sup>
A	10.67 $\pm$ 2.27	1.14 $\pm$ 0.28	0.50 $\pm$ 0.20	0.60 $\pm$ 0.11	0.31 $\pm$ 0.25	2.44 $\pm$ 0.49
B	13.89 $\pm$ 1.95*	5.13 $\pm$ 1.24*	1.78 $\pm$ 0.70*	0.91 $\pm$ 0.17	3.41 $\pm$ 1.44*	3.26 $\pm$ 0.52*
C	16.91 $\pm$ 3.05	6.79 $\pm$ 1.16	2.18 $\pm$ 0.66	0.84 $\pm$ 0.14	4.96 $\pm$ 1.39	3.33 $\pm$ 0.55*
D	13.54 $\pm$ 2.69*	4.47 $\pm$ 0.92*	1.62 $\pm$ 0.49*	0.90 $\pm$ 0.15	2.83 $\pm$ 0.83*	3.11 $\pm$ 0.62*
E	18.43 $\pm$ 5.10	7.51 $\pm$ 0.88	2.73 $\pm$ 0.79	0.88 $\pm$ 0.12	5.39 $\pm$ 1.04	4.08 $\pm$ 0.61
F	14.03 $\pm$ 2.48 <sup>#</sup>	6.36 $\pm$ 1.31 <sup>#</sup>	2.12 $\pm$ 0.71	1.06 $\pm$ 0.18	4.33 $\pm$ 1.45 <sup>#</sup>	3.03 $\pm$ 0.66
G	16.12 $\pm$ 3.31	7.34 $\pm$ 1.28 <sup>#</sup>	2.33 $\pm$ 0.62	1.01 $\pm$ 0.14	5.27 $\pm$ 1.30 <sup>#</sup>	3.14 $\pm$ 0.60
H	13.18 $\pm$ 3.00 <sup>#</sup>	5.64 $\pm$ 1.37 <sup>#</sup>	1.88 $\pm$ 0.46 <sup>#</sup>	1.10 $\pm$ 0.18	3.69 $\pm$ 1.51 <sup>#</sup>	3.00 $\pm$ 0.62 <sup>#</sup>
I	19.10 $\pm$ 3.24	9.16 $\pm$ 1.27	2.64 $\pm$ 0.56	0.99 $\pm$ 0.21	6.97 $\pm$ 1.16	3.75 $\pm$ 0.90

Data are presented as mean $\pm$ SD,  $n=8$  in each group. \* $P < 0.05$  versus Group E; <sup>#</sup> $P < 0.05$  versus Group I. TC: Total cholesterol; TG: Triglyceride; HDL: High density lipoprotein; LDL: Low density lipoprotein; Ca<sup>2+</sup>: Serum calcium; SD: Standard deviation



**Figure 2:** Aortic intima thickness and blood lipid indices of rats in each group. (a) Aortic intimal thickness in each group. (b) Serum total cholesterol level in each group. (c) Serum triglyceride level in each group. (d) Serum high density lipoprotein-cholesterol level in each group. (e) Serum low density lipoprotein-cholesterol level in each group. (f) Serum Ca<sup>2+</sup> level in each group. Data are presented as mean ± standard deviation, *n* = 8 in each group. \**P* < 0.05 versus Group E; #*P* < 0.05 versus Group I

groups was significantly lower than that in the saline group (Group I) (*P* < 0.05 in ANOVA and *post hoc*).

**Triglyceride**

In the liver injury groups, TG level in the atorvastatin (Group B) and atorvastatin combined with PNS (Group D) groups was significantly lower than that in the saline group (Group E) (*P* < 0.05). TG level was not significantly different between PNS (Group C) and saline (Group E) groups. In the nonliver injury groups, TG level in the atorvastatin combined with PNS group (Group H) was significantly lower than that in the saline group (Group I) (*P* < 0.05 in ANOVA and *post hoc*); TG level in the atorvastatin (Group F) and PNS (Group G) groups were not significantly different from that in the saline group (Group I).

**High-density lipoprotein-cholesterol**

HDL-C level in the medication-administered groups was higher than that in the saline group, although the difference was not obvious. HDL-C level in the liver injury groups was lower than that in the nonliver injury groups, but the difference was not significant.

**Low-density lipoprotein-cholesterol**

In the liver injury groups, LDL-C level in the atorvastatin (Group B) and atorvastatin combined with PNS (Group D) groups was significantly lower than that in the saline group (Group E) (*P* < 0.05). LDL-C level was not significantly different between PNS (Group C) and saline (Group E) groups. In the nonliver injury groups, LDL-C level in the atorvastatin (Group F), PNS (Group G), and atorvastatin combined

with PNS (Group H) groups was significantly lower than that in the saline group (Group I) ( $P < 0.05$  in ANOVA and *post hoc*).

### Ca<sup>2+</sup>

In the liver injury groups, Ca<sup>2+</sup> content in the atorvastatin (Group B), PNS (Group C), and atorvastatin combined with PNS (Group D) groups was significantly lower than that in the saline group (Group E) ( $P < 0.05$ ). In the nonliver injury groups, Ca<sup>2+</sup> in the atorvastatin combined with PNS group (Group H) was significantly lower than that in the saline group (Group I) ( $P < 0.05$  in ANOVA and *post hoc*); Ca<sup>2+</sup> content in the atorvastatin (Group F) and PNS (Group G) groups was not significantly different from that in the saline group (Group I).

## Liver function indices of rats in each group

Liver function indices of rats in each group were showed in Table 2 and Figure 3.

### Alanine aminotransferase

In the liver injury groups, ALT level in the atorvastatin group (Group B) was significantly higher than that in the saline group (Group E) ( $P < 0.05$ ). ALT level in the PNS (Group C) and atorvastatin combined with PNS (Group D) groups was significantly lower than that in the saline group (Group E). In the nonliver injury groups, ALT level in the medication-administered groups (Groups F, G, and H) was not significantly different from that in the saline group (Group I).

### Aspartate aminotransferase

In the liver injury groups, AST level in the atorvastatin group (Group B) was significantly higher than that in the saline group (Group E) ( $P < 0.05$ ). AST level in the PNS (Group C) and atorvastatin combined with PNS (Group D) groups was significantly lower than that in the saline group (Group E). In the nonliver injury groups, AST level in the medication-administered groups (Groups F, G, and H) was not significantly different from that in the saline group (Group I).

### Alkaline phosphatase

In the liver injury groups, ALP level in the PNS (Group C) and atorvastatin combined with PNS (Group D) groups was significantly lower than that in the saline group (Group E) ( $P < 0.05$ ). ALP level was not significantly different between atorvastatin (Group B) and saline (Group E) groups. In the nonliver injury groups, ALP level was not significantly different between medication-administered groups (Groups F, G, and H) and saline group (Group I).

### Total bilirubin

In the liver injury groups, TBIL level in the atorvastatin group (Group B) was significantly higher than that in the saline group (Group E) ( $P < 0.05$ ). TBIL level in the PNS (Group C) and atorvastatin combined with PNS (Group D) groups was significantly lower than that in the saline group (Group E). In the nonliver injury groups, TBIL level was not significantly different between medication-administered groups (Groups F, G, and H) and saline group (Group I).

### r-glutamyltransferase

In the liver injury groups, r-GT level in the atorvastatin group (Group B) was significantly higher than that in the saline group (Group E) ( $P < 0.05$ ). r-GT level in the PNS (Group C) and atorvastatin combined with PNS (Group D) groups was significantly lower than that in the saline group (Group E). In the nonliver injury groups, r-GT level was not significantly different between medication-administered groups (Groups F, G, and H) and saline group (Group I).

## DISCUSSION

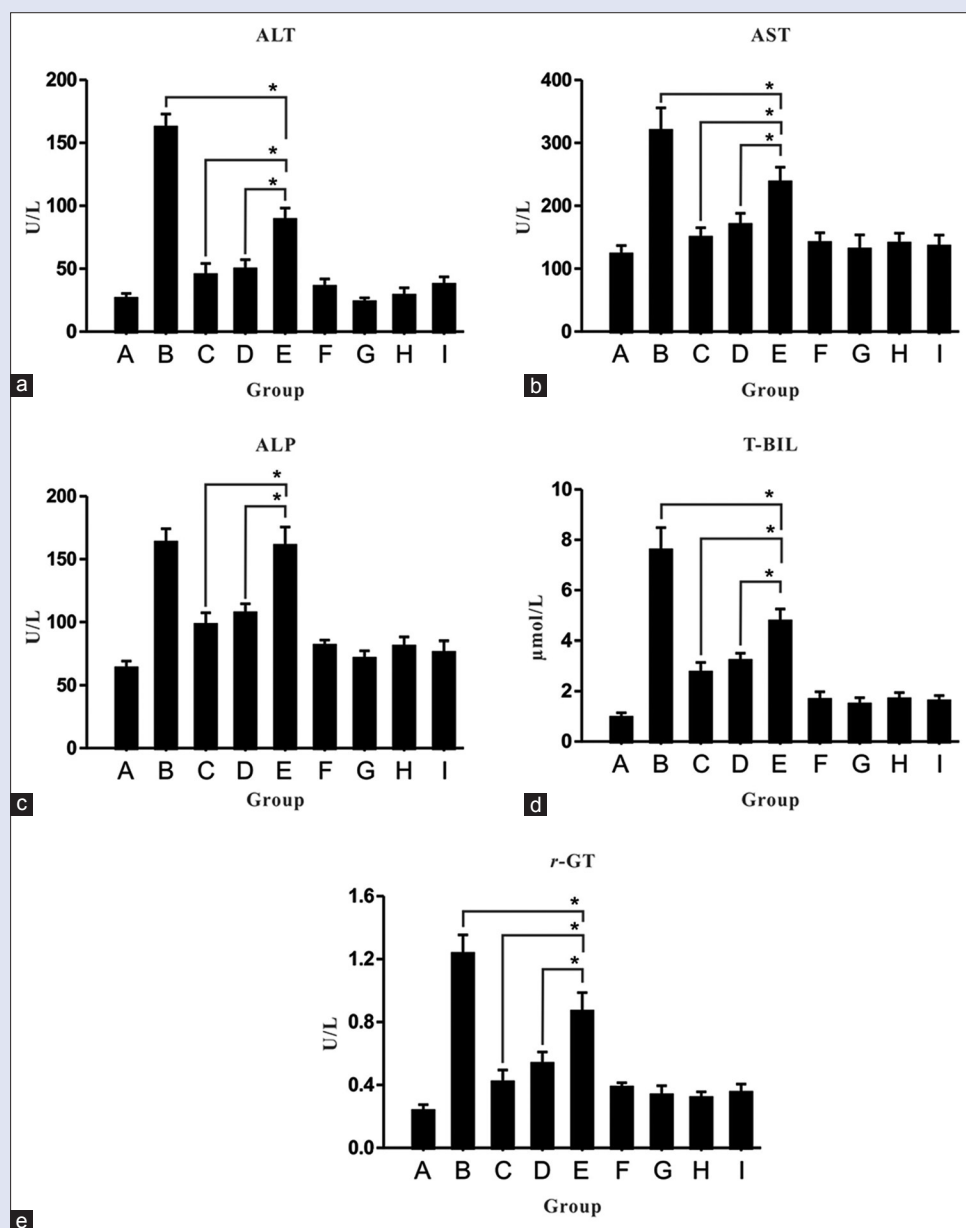
Current medical treatment of AS mainly includes antiplatelet therapy, intensive statins therapy and antihypertensive therapy.<sup>[30]</sup> Intensive statins therapy is important in stabilizing plaque, inhibiting plaque progression, and even preventing plaque rupture and occurrence of atherosclerotic events.<sup>[31]</sup> A large number of clinical trials have shown that statins can reduce 20%–40% of TC, reduce 20%–60% of LDL and reduce 7%–30% of TG.<sup>[32]</sup> Results of the SAMMPRIS study also suggest that the use of intensive statins therapy to decrease LDL-C to 1.8 mmol/L is beneficial in reducing atherosclerotic events.<sup>[33]</sup> However, the study found that although a large dose of statins is used in most patients, LDL-C still cannot be reduced to the “ideal level” of  $< 1.8$  mmol/L (70 mg/dL). Over the past 10 years, a large number of clinical studies have been done to assess the safety and efficacy of statins with long-term use, and the determined increase in liver enzymes and muscle damage caused by statins has restricted its application to a certain extent.<sup>[12,34-36]</sup> Current reports of increased risk of type 2 diabetes with statins use are of concern.<sup>[37]</sup> The most commonly reported hepatic adverse effect of statins is transaminitis, in which liver enzyme levels are elevated in the absence of proven hepatotoxicity. This class effect is usually asymptomatic, reversible, and dose related.<sup>[11]</sup>

*P. notoginseng*, which belongs to the *Araliaceae* species and *Panax* genus, is a traditional medicine that has been used in China for thousands of years. Yunnan is one of the original areas of *P. notoginseng*. PNS are the main active components purified from *P. notoginseng*, which contains a variety of monomer saponins, such as notoginsenoside R1, ginsenoside

**Table 2:** Liver function indices of rats in each group

Group	ALT (U/L)	AST (U/L)	ALP (U/L)	T-BIL (μmol/L)	r-GT (U/L)
A	26.11±10.70	122.58±35.02	63.47±14.30	0.95±0.49	0.23±0.10
B	162.10±26.41*	319.02±90.23*	163.03±27.57	7.58±2.22*	1.23±0.29*
C	44.90±22.97*	149.08±39.41*	97.80±23.59*	2.73±1.01*	0.42±0.19*
D	49.44±19.06*	169.43±45.76*	106.95±18.61*	3.21±0.75*	0.53±0.19*
E	88.69±23.42	237.05±60.23	160.58±36.87	4.76±1.22	0.87±0.29
F	35.50±15.87	140.67±40.77	81.43±10.54	1.65±0.80	0.38±0.08
G	23.38±8.82	130.58±57.24	71.00±15.84	1.47±0.68	0.33±0.15
H	28.66±15.24	139.77±41.12	80.68±18.54	1.68±0.65	0.32±0.10
I	37.32±15.29	135.12±44.79	75.77±23.09	1.60±0.57	0.35±0.14

Data are presented as mean±SD,  $n=8$  in each group. \* $P < 0.05$  versus Group E. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; T-BIL: Total bilirubin; r-GT: r-glutamyltransferase; SD: Standard deviation



**Figure 3:** Liver function indices of rats in each group. (a) Serum alanine aminotransferase level in each group. (b) Serum aspartate aminotransferase level in each group. (c) Serum alkaline phosphatase level in each group. (d) Serum total bilirubin level in each group. (e) Serum r-glutamyltransferase level in each group. Data are presented as mean  $\pm$  standard deviation,  $n = 8$  in each group. \* $P < 0.05$  versus Group E

Rb1 and ginsenoside Rg1.<sup>[38]</sup> Attempts by the low-income group, particularly the rural dwellers in the developing countries, in the face of the scarce socioeconomic resources, have led more people opting for herbal therapeutics.<sup>[39]</sup> With the development of natural plant medicine industry, more and more natural products are being clinically used worldwide. Currently, PNS are used for the treatment of cardiovascular diseases due to its good vasodilatory, blood lipid regulatory, and anti-inflammatory actions. PNS are now being developed into different pharmaceutical formulations. Some of these agents have been used in the clinic for decades.

PNS are considered a good source of leading compounds for novel antiplatelet and anticoagulant therapeutics, which play important roles in the treatment of thrombosis. PNS can improve the content of platelet CAMP, reduce the generation of thromboxane A<sub>2</sub>, inhibit the release of promote platelet aggregation active substance such as Ca<sup>2+</sup>, 5-HT,

play the role of anti-platelet aggregation.<sup>[40]</sup> The mainly antiplatelet component of PNS is represented by Rg1. PNS also can reduce the blood viscosity, increase the erythrocyte deformability, reduce the effect of human plasma coagulation factor I,<sup>[41]</sup> which has important value for the prevention of thrombosis.

He Xuefeng showed that PNS could prevent and control experimental AS in rabbits by regulating lipid metabolism, antioxidation, and scavenging free radicals.<sup>[42,43]</sup> In recent years, PNS have demonstrated a good curative effect in the treatment of hepatitis, hepatic fibrosis, cirrhosis, and other liver disease.<sup>[44-46]</sup> PNS mainly affect the liver function in the early stage of liver cell degeneration and necrosis, significantly in the change of ALB, ALT, AST, and so on. PNS significantly increase superoxide dismutase content in serum and liver tissue, reduce liver glycogen consumption, improve microcirculation in the liver, and reduce damage to organelles such as mitochondria and endoplasmic

reticulum, so as to confer a protective effect to the liver. PNS reportedly reduced serum ALT, AST, and LDH activities in a mice liver injury model, hence capable of reducing degeneration and necrosis of liver cells.<sup>[47]</sup>

In this study we established rat models for AS combined with liver injury and without liver injury, respectively. We then compared the effects of atorvastatin and PNS on the regulation of blood lipids, regulation of immune inflammation, and endothelial function. The effect of PNS combined with atorvastatin as treatment for liver injury and nonliver injury in the model groups was investigated. The effect of combination therapy on the function of lowering blood lipid, stable atherosclerotic plaque, and liver function improvement was assessed.

In the experimental results, the single use of PNS in the rat model for AS significantly reduced Ca<sup>2+</sup> content in serum, whereas the effect of lowering TC, TG, and LDL-C is not apparent, especially as compared with atorvastatin treatment. PNS combined with atorvastatin treatment of the rat model for AS displayed a noticeable, synergistic effect that allowed for better reduction of TC, TG, LDL-C, and Ca<sup>2+</sup> in the serum than that with the single use of PNS or atorvastatin.

In the rat liver injury combined with AS model, the single use of PNS significantly improved liver function, whereas atorvastatin alone only aggravated liver injury in the rat model. The effect of PNS combined with atorvastatin on liver function was significantly better than that of atorvastatin alone. The combined use of PNS and atorvastatin showed good stability of liver function on the liver injury combined with AS model.

## CONCLUSION

Results from our current study show that PNS exhibited lipid-lowering and liver-stabilizing functions in the rat model of liver injury combined with AS, but the better treatment effect was demonstrated by the PNS and atorvastatin combination, suggesting that PNS may have a different mechanism of action from the existing lipid-lowering medicine. As an auxiliary lipid-lowering and liver-stabilizing medicine, PNS may be used in combination with other lipid-lowering agents to produce a synergistic effect and reduce the liver damage side effect by the latter.

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## Conflicts of interest

There are no conflicts of interest.

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