Effects of *Polygonatum sibiricum* Polysaccharide on Antioxidant Capacity of the Liver in High-fat Diet Rats

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Abstract [**Objectives**] This study was conducted to investigate the effects of *Polygonatum sibiricum* polysaccharide (PSP) on antioxidant function in high-fat diet obese rats. [**Methods**] Thirty five healthy male SD rats were selected to establish an obesity model after feeding a high-fat for 8 weeks. They were then randomly divided into a normal group (NC), a high-fat diet group (HF), and an HF + *P. sibiricum* polysaccharide group [HF + PSP, 300 mg/(kg · d)]. After 6 weeks of PSP intervention, the serum and liver of rats were collected, and the activity of aspartate transaminase (AST) and alanine aminotransferase (ALT) in serum, the enzyme activity of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) in liver tissue were measured. The pathological changes of liver tissue were observed by HE staining. [**Results**] Compared with the HF group, PSP could effectively inhibit obesity caused by high-fat diet. It reduced body weight and serum AST and ALT levels, increased the contents of T-SOD, CAT and GSH-Px in the liver, and inhibited the accumulation of MDA content, thereby reducing damage to liver cells caused by a high-fat diet. It indicated that PSP could effectively inhibit obesity in high-fat diet rats and enhance their antioxidant capacity. [**Conclusions**] This study provides a reference for the study of the antioxidant capacity of PSP.

Key words *Polygonatum sibiricum* polysaccharide; High-fat diet; Obesity; SD rat
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Obesity is a global epidemic caused by multiple factors. According to relevant reports, it is estimated that by 2025, more than half of global deaths will be caused by obesity and related diseases[1]. At present, the main methods for treating obesity include changing lifestyle habits, increasing exercise, medication treatment, and weight loss surgery, but the effects are not satisfactory, especially for Western medicine treatment, which can cause serious side effects[2]. Therefore, it is particularly important to develop safe and effective natural products to treat obesity. As a kind of natural product, the function of plant polysaccharides has become a research hotspot in recent years. Numerous literature studies have found that polysaccharides have antioxidant[3], anti-tumor[4], liver-protection[5] and anti-obesity effects[6]. Several kinds of natural polysaccharides have been proven to play important roles in clearing free radicals, preventing oxidative damage, and inhibiting obesity, and can serve as new potential antioxidants[7–9].

Polygonatum sibiricum polysaccharide (PSP) is one of the main active ingredients of *P. sibiricum*, which has been widely used in clinical treatment of diabetes[10], osteoporosis[11] and atherosclerosis[12]. It has functions such as immune-regulation, anti-tumor, anti-fatigue, and antioxidant[13–15]. At present, research on the antioxidant properties of PSP mainly focuses on *in-vitro* antioxidant activity[16–17] and *in-vivo* aging models[4,15], but there are few reports on the oxidative stress induced by obesity caused by high-fat diet. Therefore, in this study, with SD (sprague dawley) male rats with oxidative stress induced by obesity due to high-fat diet as the object of study, the effects of PSP on the activity of aspartate transaminase (AST) and alanine aminotransferase (ALT) in serum, the enzyme activity of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-P) and malondialdehyde (MDA) content in liver tissue and pathological sections of the liver, aiming to provide reference for the research on antioxidant capacity of PSP.

Materials and Methods

Animals

Thirty SPF male healthy SD rats (body weight: 250 – 280 g), purchased from Hunan SJA Laboratory Animal Co., Ltd. (license number: SCXK (Xiang) 2016-0002); ordinary feed, provided by Hunan SJA Laboratory Animal Co., Ltd. (10% kcal fat, 3.6 kcal/g); high-fat feed, provided by Beijing Botai Hongda Biotechnology Co., Ltd. (34% kcal fat, 4.6 kcal/g).

Drugs and reagents

P. sibiricum polysaccharide (PSP) was separated and purified from *P. sibiricum*. Adopting the alkali-extraction and alcohol-precipitation method, the PSP was obtained through Sevag separation and purification. PSP is mainly composed of five monosaccharides, namely galactose, arabinose, rhamnose, xylose and glucose. The total sugar content in the sample was measured to be about 51.66% by the phenol-sulfuric acid method[18].

Superoxide dismutase (SOD) kit, catalase (CAT) kit, malondialdehyde (MDA) kit, glutathione peroxidase (GSH-Px) kit, alanine aminotransferase (ALT) kit, and aspartate transaminase (AST) kit were all purchased from Nanjing Jiancheng Bioengineering

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Methods

Establishment of rat obesity model Thirty five male SD rats were randomly divided into two groups: a normal group (NC, $n = 10$) and a high-fat diet group (HF, $n = 25$). The NC group was fed on a normal diet and the HF group had a high-fat diet. After 8 weeks of feeding, 20 rats with an average body weight exceeding 20% of the average body weight of the NC group, were selected from the HF group. The selected rats were re-divided into an HF group and an HF + PSP group, with 10 animals in each group.

Drug administration Next, the HF + PSP group was administered at a dose of 300 mg/kg[18]. For the NC and HF groups, corresponding volumes of normal saline were administered by gavage. After 6 weeks of administration, rats stayed overnight on an empty stomach, and all rats were anesthetized with pentobarbital sodium, and blood was extracted from the abdominal aorta to separate serum for centrifugation. The bodies were dissected to separate the liver and perirenal fat, which were stored in a refrigerator at −80 ℃ for later use.

Body weight Before and after modeling, the weights of rats were measured once a week and recorded.

Effects on liver function According to the instructions of the reagent kit, the levels of ALT and AST in serum were measured using an automatic biochemical analyzer.

Liver oxidation indexes Firstly, a liver tissue homogenate was prepared according to the ratio of liver : physiological saline = 1 : 9. The obtained homogenate was centrifuged to get a supernatant, which was determined for SOD, CAT and GSH-Px enzyme activity and MDA content according to the instructions of the reagent kit.

Pathological analysis of liver tissue First, the liver tissue was fixed with formalin, and then eluted with gradient ethanol. Next, the tissue was transparentized with xylene and embedded with paraffin. The embedded paraffin blocks were cut into pieces approximately 5 μm thick. Finally, the pieces were dehydrated with ethanol solutions of different concentrations, and re-stained with an eosin solution and transparentized with xylene. The prepared sections were observed under an optical microscope for the staining conditions, and their degree of lipid accumulation was evaluated.

Statistical methods

SPSS 21.0 software was used for significance analysis, and the experimental results were presented in the form of ($\bar{x} \pm s$). The comparison of the mean values between the two groups of samples was conducted by an independent-samples t test, with $P < 0.05$ being significant and $P < 0.01$ being extremely significant.

Results and Analysis

Effects of PSP on body weight of rats fed a high-fat diet

As shown in Fig. 1, after 8 weeks of high-fat diet, the weight of rats in the HF group was (620.3 ± 7.32) g, significantly higher than that in rats of the NC group by 20.23%. After 6 weeks of treatment with PSP, the weight gain of rats in the HF + PSP group significantly decreased ($P < 0.01$). It indicated that PSP could inhibit the formation of obese rats with a high-fat diet.

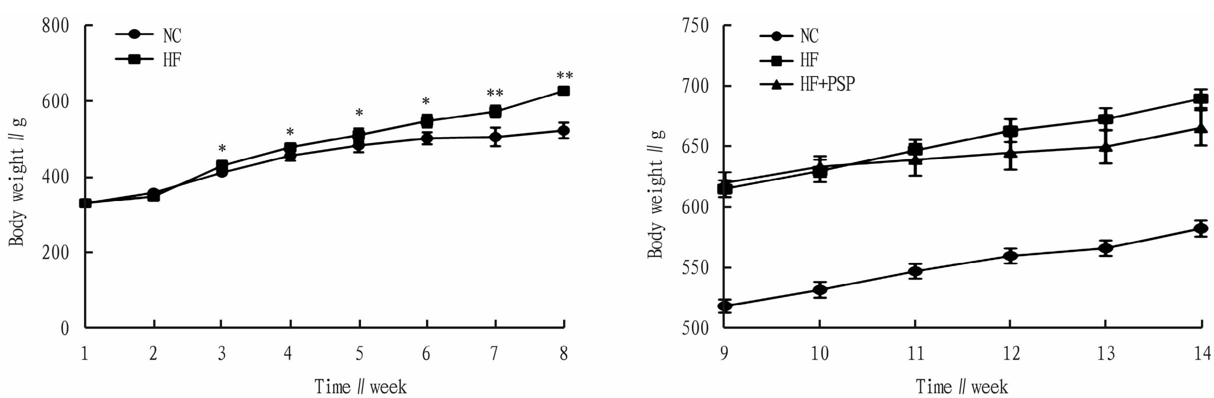

Fig. 1 Effects of PSP on body weight of rats ($\bar{x} \pm s$, $n = 10$)

Effects of PSP on liver function enzymes of rats fed a high-fat diet

Fig. 2 shows that a high-fat diet significantly induced an increase in ALT and AST levels in rats of the HF group ($P < 0.01$), and after 6 weeks of intervention with PSP, the increase in serum ALT and AST levels caused by a high-fat diet could be significantly reduced ($P < 0.01$). It indicated that PSP alleviated liver function damage caused by a high-fat diet.

Effects of PSP on activity of antioxidant enzymes in the liver of rats fed a high-fat diet

As shown in Fig. 3, compared with the NC group, the activity of antioxidant enzyme (CAT, SOD, and GSH-Px) of the HF group decreased significantly ($P < 0.01$), while the MDA content significantly increased ($P < 0.01$). After 6 weeks of intervention with PSP, the activity of antioxidant enzymes in high-fat diet rats significantly increased ($P < 0.01$), and the MDA content in the liver decreased ($P < 0.01$). It indicated that PSP enhanced the antioxidant capacity of rats fed a high-fat diet.

Effects of PSP on liver pathological sections of obese rats fed a high-fat diet

Fig. 4 shows that compared with the normal group, the liver sections of rats in the model group showed significant vesicular degeneration and a significant increase in lipid droplets. With the increase of intervention dose of PSP, vesicular degeneration was improved, and lipid droplets in the liver of rats were reduced, and cell arrangement became more orderly. It further showed that PSP alleviated liver function damage caused by high-fat diet.

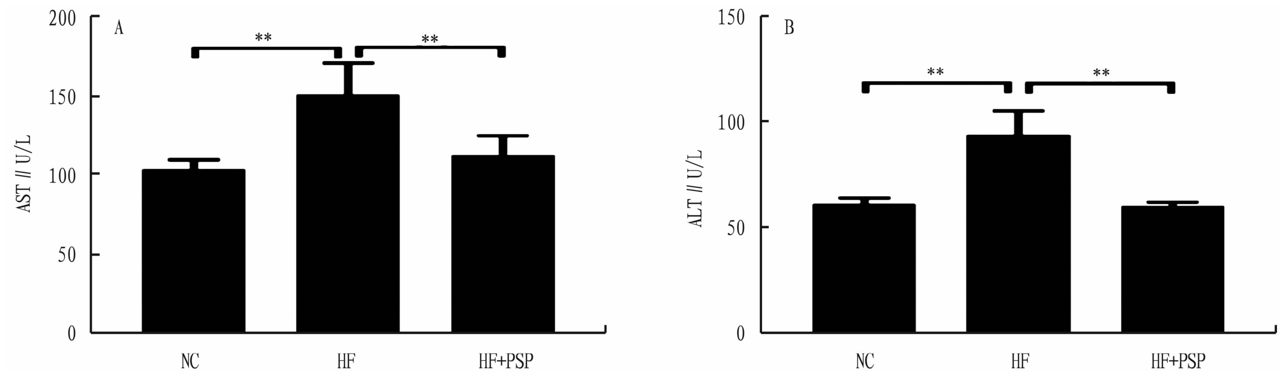

Fig. 2 Effects of PSP on ALT and AST levels in rats ($\bar{x} \pm s$, $n=10$)

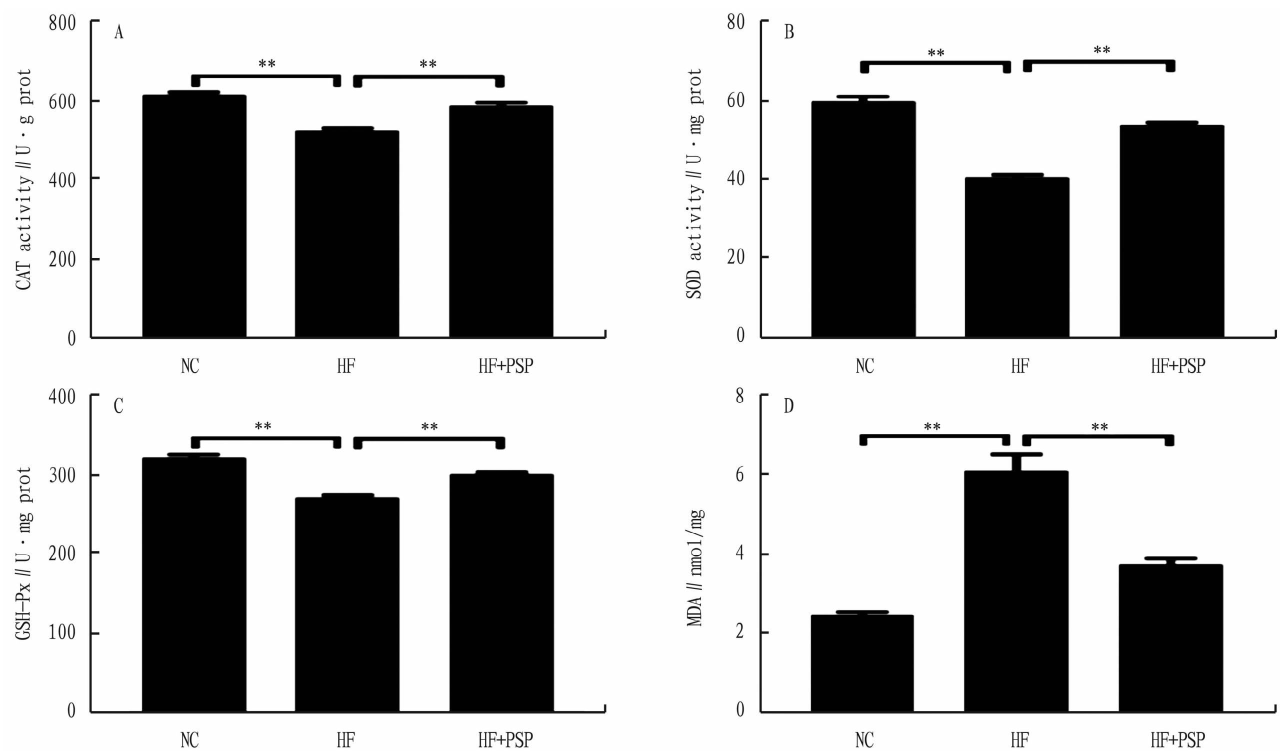

Fig. 3 Effects of PSP on antioxidant levels in the liver of rats

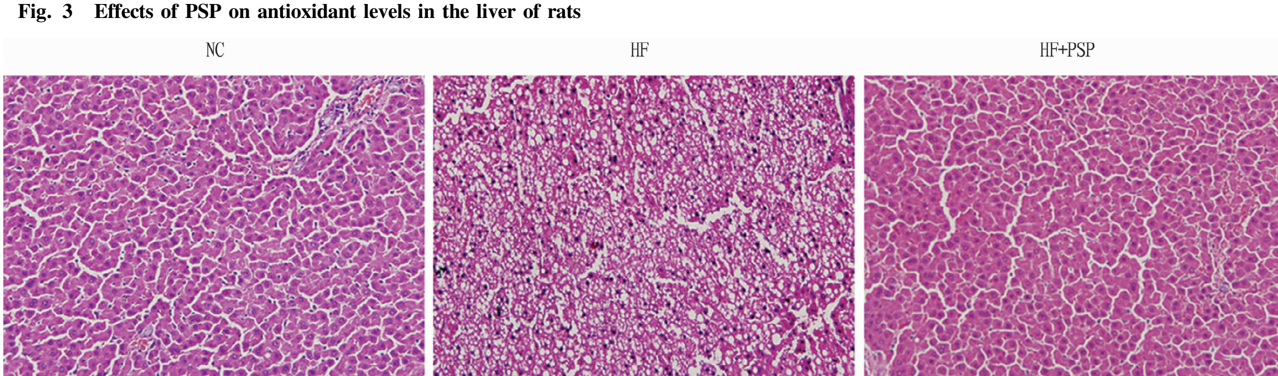

Fig. 4 Effect of PSP on liver histopathology in rats (HE, ×100)

Discussion and Conclusions

As a chronic metabolic disease, obesity is prone to cause oxidative stress, which eventually induces various metabolic disorders such as diabetes, cardiovascular disease and cancer. Some studies have shown that long-term high-fat diet will lead to the increase of reactive oxygen species in the body, causing a series of metabolic disorders, and the pathological mechanisms of these metabolic disorders can be understood as being caused by oxidative stress injury induced by high-fat diets[19]. The results of this study further prove that a high-fat diet is easy to cause oxidative damage. At present, the research on PSP mainly focuses on their extraction, separation and purification, and blood sugar- and lipid-lowering, antioxidant, immune-regulation and anti-tumor effects[3-6]. However, more theoretical basis is needed for the study of their effects on liver oxidative stress caused by obesity induced by high-fat diet. The results of this study indicated that PSP had a significant improvement effect on oxidative damage caused by high-fat diet.

Oxidative damage refers to the biomembrane lipid peroxidation reactions induced by excessive accumulation of ROS in the body caused by imbalance between the production and elimination of reactive oxygen species, which is closely related to the occurrence and development of various diseases. MDA is an important indicator to measure the degree of lipid peroxidation, which can reflect the degree of membrane lipid peroxidation in organisms. The liver is the main organ attacked by reactive oxygen species. In the process of oxidative damage to the liver, lipid peroxidation is produced due to the action of free radicals, and MDA leads to the disorder of biomacromolecules, including nucleic acids, proteins, phospholipids, *etc.* SOD and CAT are essential enzymes scavenging oxygen free radical in the body, which further convert superoxide anion free radical O^{2-} into H_2O_2 and O_2, and H_2O_2 was then decomposed into water by CAT or GSH-Px, preventing oxidative stress damage[20-21]. Moreover, the activity of AST and ALT can sensitively reflect the degree of liver cell damage. In this study, obesity of rats induced by high-fat diet resulted in a significant increase in liver MDA content and AST and ALT activity, while SOD, CAT and GSH-Px activity significantly decreased, indicating that high-fat diet caused oxidative damage to the liver of rats, but intervention with PSP significantly reduced MDA content and AST and ALT activity and increased the activity of antioxidant enzyme systems SOD, CAT, and GSH-Px, indicating that PSP could alleviate oxidative damage to the liver caused by high-fat diet, which is similar to the study by Deng *et al.*[22] and Liang *et al.*[23] on the anti-liver injury effect of polysaccharides. It might be due to the fact that polysaccharides mainly play an anti-liver injury role by resisting free radical damage, combating liver cell calcium overload and regulating mitochondrial function.

In addition, HE staining revealed vesicular degeneration in the liver of rats in the high-fat diet group, accompanied by a significant increase in lipid droplets. After intervention with PSP, the deposition of fat in the liver of rats was reduced, resulting in a more orderly arrangement of cells.

Overall, it indicated that PSP could improve liver oxidative damage, enhance antioxidant capacity and reduce fat deposition in the liver by reducing AST and ALT levels, thereby inhibiting the formation of obesity in rats fed a high-fat diet. In the future, the molecular mechanism by which PSP can alleviate liver oxidative damage will be further clarified.

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carried out according to changes in the weather and the occurrence laws of diseases and pests, and timely prevention and control measures are taken.

Conclusions

Shiyan is a suitable cultivation area for olive trees. In recent years, the local municipal party committee and government have attached great importance to the development of the olive industry, and have included olive as a key support direction for the development of woody oil crops in Shiyan City. With the rapid development of the olive industry in Shiyan City, more and more olive orchards will need to be transplanted with large trees. Transplanting big trees is a complex project, and its season, climate and age can directly affect the survival rate of transplanted trees. However, as long as the transplanting measures are scientific and reasonable, and the trees are transplanted according to the process described earlier, and scientifically nurtured and managed in the later stage, the survival rate of transplanted big olive trees can be effectively improved, and the fruiting cycle can be shortened, thereby achieving early results.

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