

Evaluation of the anti-fatigue activity of Schisandra chinensis polysaccharides

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Abstract

Schisandra chinensis is a medicative and food plant in China, wealthy during a variety of functional components and wealthy during pharmacological activities. The specific aim of this study was to evaluate the anti-fatigue properties of *Schisandra chinensis* polysaccharides (SCP). It was first structurally characterized using FTIR and UV, followed by weight-loaded swimming test and determination of several fatigue-related biochemical indices in mice after exhaustion to evaluate the anti-fatigue ability of SCP. The results showed that SCP is a protein-bound polysaccharide, and it significantly prolonged swimming time, increased liver glycogen and muscle glycogen content, decreased lactate (LA), Blood urea nitrogen (BUN), malondialdehyde (MDA) levels, and increased glutathione peroxidase (GSH-PX), superoxide dismutase (SOD) and catalase (CAT) activities. Pearson correlation analysis showed that there was a good correlation between the in vivo anti-fatigue effect and antioxidant activity of SCP. Therefore, SCP can be applied as a potential anti-fatigue nutritional supplement in functional foods for the relief of exercise-related fatigue.

1. Introduction

Fatigue is a physiological change in the body caused by energy expenditure during work and activity. Workers experience fatigue and reduced work function after a sustained period of work. Fatigue is also a natural protective reflection of the human collective to avoid damage[1, 2]. Studies have shown that fatigue is mainly divided into central fatigue and peripheral fatigue, and that a continuous supply of energy to the body and removal of metabolites can effectively alleviate peripheral fatigue[3]. In addition, high-intensity exercise causes excessive production of reactive oxides and malondialdehyde, which in turn can lead to oxidative stress[4]. The body can develop symptoms of fatigue. More research has found that many medicinal plants are in the spotlight for their research and application in relieving exercise-induced fatigue due to their good efficacy and fewer side effects[5].

Schisandra is the dried ripe fruit of *Schisandra chinensis* (Turcz.) Baill or *S. Sphenanthera* Rehd. et Wils in the family Magnoliaceae (Fig. 1). The former is customarily referred to as 'northern schisandra' and the latter as 'southern schisandra'. The former is commonly referred to as "BeiWuWeiZi" and the latter as "NanWuWeiZi"[6, 7]. In the Xinxu Ben Cao of the Tang Dynasty, it is written that "the skin and flesh of Schisandra are sweet and sour, the core is hard, and all have a salty taste." Schisandra is a traditional Chinese herb with abundant resources and a very wide distribution, mainly in Heilongjiang, Jilin, Liaoning, Inner Mongolia, Hebei, Shanxi, Ningxia, Gansu and Shandong in China, and in countries such as Japan, North Korea and Russia [8]. The main active ingredients of *Schisandra chinensis* are lignans, polysaccharides, volatile oils, phenolic acids, triterpenoids, and flavonoids [9–13].

As a medicinal and food product, schisandra is mostly used in the field of medicine. In some recent studies, it has been found that lignans, the active ingredients in a variety of schisandra seeds, have superior neuroprotective, hepatic and renal cellular and inflammation-relieving effects[14–17], while polysaccharides have strong antioxidant, immunomodulatory, hypoglycaemic and lipid-lowering

properties[18–22]. Multiple effects give Schisandra a wide range of applications. To our best knowledge, few researches concerning the potential anti-fatigue property of Schisandra chinensis were published yet. Therefore, this study investigated the anti-fatigue effect of Schisandra chinensis, aiming to provide an experimental basis for further development of Schisandra chinensis as a novel anti-fatigue agent.

2. Materials And Methods

2.1 Raw materials and reagents

Schisandra chinensis was purchased from Hongpin Tang Specialty Trading Company, Songjianghe Town, Fusong County, Baishan City, Jilin Province, China, and stored at room temperature before use. Assay kits for the determination of blood lactate (LA), liver/muscle glycogen (LG, MG), and cystathione peroxidase (GSH-Px) were purchased from Nanjing Jiancheng Institute of Biological Engineering (Nanjing, Jiangsu Province, China). Blood urea nitrogen (BUN), superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) were purchased from Beijing Solarbio Science & Technology Co., Ltd. All other reagents used in this study were analytically pure.

2.2 Preparation of polysaccharides

The dried fruits of Schisandra are washed with water to remove impurities. They were heated at 70°C for 24 hours to constant weight, then crushed and passed through a 40 mesh sieve. Weigh 500 g of powdered Schisandra chinensis and soxhlet extract with petroleum ether (boiling range 30-60°C) for 6 h. At the end of the extraction, the extract with distilled water (1:10, w:v) at 90°C for 3 h. Filter and extract the precipitate (1:8, w:v) for 2 h. Combine the filtrates and concentrate to 1/10 of the original volume, then add 4 times the volume of 95% EtOH overnight at 4°C. The precipitate was centrifuged, washed sequentially with anhydrous ethanol, acetone, and anhydrous ether, and then redissolved in hot water. The precipitate was deproteinised with sevrage reagent. The precipitate was dialyzed against tap water and deionized water for 24 hours to remove small molecules and pigments, respectively, and then freeze-dried to obtain Schisandra crude polysaccharide, named SCP.

2.3 General analysis

SCP sugar content was determined by the phenol sulfate photometric method, and the standard curve was prepared using glucose as standard ($y=0.0127x-0.0544$, $R^2=0.9993$). SCP was mixed with 200mg of KBr powder, well ground in an agate mortar, pressed into tablets, and then scanned by Fourier Transform Infrared (FTIR) in the wavelength range of 4000 to 400 cm^{-1} . SCP was dissolved in ultrapure water and scanned by UV-Vis spectroscopy in the wavelength range of 200-700 nm.

2.4 Animal and experimental design

Forty Kunming male mice (KM, 4-6 weeks old, Specific Pathogen Free (SPF) class) were purchased from Beijing Viton Lihua Laboratory Animal Technology Co. 40 ICR mice were housed in cages (5 each) with

controlled temperature ($25\pm 1^{\circ}\text{C}$), relative humidity (50%-60%), and lighting (12 h light/dark cycle). Mice were allowed to drink and eat freely. After one week of acclimatization feeding, mice were randomly divided into five groups (8 mice per group), including negative control group (NCG); positive control group (PCG); low dose group (SCP-L); medium dose group (SCP-M); and high dose group (SCP-H), and the three experimental groups were administered by gavage at a dose of 100, 200, and 300 mg/kg, respectively, and the same volume of NCG was given distilled water, and the NCG was gavaged with *Rhodiola rosea* oral solution. SCP was administered by noninvasive feeding injection at 5 p.m. daily for 28 days. The mice were weighed every 4 days during the 28-day gavage period. The acute toxicity of SCP was assessed by evaluating changes in body weight and mortality during gavage. The study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals.

2.5 Weight-loaded swimming test

After a final gavage treatment and a 30-minute rest, mice were subjected to an exhaustion swimming experiment. Mice were tied with lead blocks (approximately 7% of their body weight) on their tails and placed in a tank (50cm x 50cm x 40cm) with a water temperature of $25\pm 2^{\circ}\text{C}$ and a water depth of 35cm. The swimming time was recorded when the mice were submerged for 10s without surfacing to breathe. During this time of the swimming test, the water in the tank was gently stirred with a glass rod so that the mice swam to exhaustion.[24].

2.6 Measurement of biochemical parameters related to fatigue

At the end of the swimming experiment, mice were rested for 30 minutes, then general anaesthesia with 1.5% pentobarbital sodium and euthanised. All serum and tissue samples were collected and biochemical parameters, including MDA, BUN, SOD, LA, CAT, GSH-PX, and liver/muscle glycogen, were measured according to product instructions to evaluate the fatigue resistance of SCP.

2.7 Hematoxylin and eosin (H&E) staining

Heart, skeletal muscle, and liver tissues from mice were collected, fixed in 10% formalin, embedded in paraffin, cut into 4- μm thick sections, stained with hematoxylin and eosin and pathologically observed under a light microscope to assess the extent of damage to various organs.

2.8 Statistical analysis

All the experimental data were expressed in the form of mean $\pm$ standard deviation (SD). The statistical analyses were conducted by oneway analysis of variance (ANOVA) and Duncan's test using software SPSS 26 (SPSS, Chicago, IL, USA). The differences were considered statistically significant at $P < 0.05$ and extremely significant at $P < 0.01$, respectively. R software version 4.12.0 was used for Pearson correlation analysis to evaluate the correlation between the indicators.

3. Results And Discussion

3.1 Physicochemical property of SCP

The powder of *Schisandra chinensis* was subjected to hot water extraction, deproteinization, and freeze-drying to obtain crude polysaccharide with a calculated yield of 1.56%. The sugar content of SCP was measured by phenol-sulfuric acid method as 52.3%. SCP is a pinkish powder that is readily soluble in water. FTIR spectroscopy of polysaccharides can provide information about the functional groups constituting the polysaccharides and has the advantages of being efficient, fast, convenient, and non-destructive to the sample. As shown in Fig. 2A, the stronger absorption peak at 3276 cm⁻¹ is caused by the stretching vibration of the hydroxyl group. 2929 cm⁻¹ absorbs the C-H stretching vibration peaks of methyl and hypomethyl groups. 1731 cm⁻¹ absorption peak is characteristic of an ester bond, indicating that the A part of galacturonic acid is in the form of a lactone. 1626 cm⁻¹ absorption peak is formed by the C=O stretching vibration of the carbonyl group. 1233 cm⁻¹ absorption peak is formed by the C=O stretching vibration of the carbonyl group. The absorption peak at 1233 cm⁻¹ generally belongs to acetyl-CH₃. In addition, the absorption peaks at 1147, 1077, and 1014 cm⁻¹ indicate that SCP contains pyranose. The absorption peak at 758 cm⁻¹ belongs to the pyranose conformation of polysaccharide. The results showed that the FT-IR spectra of SCP all had the characteristic absorption peaks of polysaccharides, indicating that the separation and purification of polysaccharides in the study did not change or destroy the chemical groups of the polysaccharides themselves. As shown in Fig. 2B, the absorption peaks at 260-280 nm in the UV spectra were those of proteins, indicating that SCP was a combination of polysaccharide and protein, and indicating that the protein could not be completely removed by the seveag method.

3.2 Effect of SCP on anti-fatigue function in mice

3.2.1 Body weight and other metabolism-related organ weights

After 4 weeks of gavage, the body weights of mice all increased. As shown in Table 1, there was no significant difference between NCG group and PCG group and SCP group ($p>0.05$). The behavioral characteristics of mice were observed, and no abnormalities or deaths were found, which indicated that gavage of SCP had no significant effect on body weight of mice, and there were no toxic side effects. The thymus, liver and kidney are important immune organs, detoxification and metabolism organs of mice. the thymus, liver and kidney indices of mice in SCP group increased compared with NCG, but there was no significant difference, so it could relieve the fatigue symptoms of mice to some extent.

Table 1 Effects of *Schisandra chinensis* on body weight and organs of mice

Body weight(g)	NCG	PCG	SCP-L	SCP-M	SCP-H
Initial weight(g)	23.28±1.96	25.12±2.16	22.60±1.25	22.85±1.62	24.65±1.21
Intermediate weight(g)	38.23±2.24	40.28±3.21	39.63±1.36	40.32±1.99	38.26±3.86
Final weight(g)	50.43±2.42	49.24±3.63	52.84±1.23	51.69±3.22	52.87±3.56
Thymus weight(g)	0.098±0.03	0.106±0.02	0.104±0.01	0.109±0.01	0.134±0.01
Liver weight(g)	2.02±0.04	2.07±0.09	2.09±0.03	2.09±0.02	2.14±0.01
Kidney weight(g)	0.54±0.01	0.62±0.02	0.60±0.01	0.62±0.04	0.68±0.01
Relative Thymus weight(%)	0.19±0.13	0.21±0.05	0.19±0.08	0.21±0.03	0.25±0.03
Relative Liver weight(%)	4.01±0.12	4.20±0.18	4.00±0.11	4.02±0.07	4.04±0.10
Relative Kidney weight(%)	1.13±0.11	1.26±0.06	1.14±0.04	1.20±0.08	1.31±0.11

3.2.2 SCP prolonged the Weight-loaded swimming time

The weight-loaded swimming test is an experimental exercise model for assessing fatigue resistance, and the duration reflects the degree of fatigue with a high degree of reproducibility. The improvement of exercise endurance is the most powerful manifestation of the anti-fatigue effect. The anti-fatigue effect of SCP was observed by measuring the duration of weight-loaded swimming in mice. As shown in Table 2, SCP was able to prolong the exercise time of mice. Compared with NCG, the swimming time of SCP group all increased, extending 34.75%, 86.87% and 149.34% respectively ($p<0.01$), and the extended time was positively correlated with the dose. The results suggest that SCP has the effect of improving exercise endurance and highlight the anti-fatigue properties of SCP.

Table 2 Effect of Schisandra chinensis on exhaustive swimming time in mice

Group	Exhausting time (min)	Extension rate (%)
NCG	13.26±1.42 ^d	—
PCG	24.65±1.90 ^b	76.22
SCP-L	17.13±1.66 ^c	34.75
SCP-M	24.00±1.67 ^b	86.87
SCP-H	32.34±2.99 ^a	149.34

3.2.3 SCP increased LG and MG

Liver glycogen is formed by the polymerization of many glucose molecules, and these glucose polymers are stored in the liver in the form of glycogen. When the body exercises or feels hungry, it can be broken down into glucose to maintain blood glucose homeostasis. The storage and breakdown of Liver glycogen can directly affect the body's ability to exercise and the duration of exercise. In addition, numerous studies have shown that physical exhaustion and muscle glycogen depletion due to exercise occur simultaneously. Therefore, the levels of Liver glycogen and muscle glycogen are important indicators of the degree of fatigue[25].

The effects of SCP on LG, MG in mice are shown in Fig3. The liver glycogen content was significantly increased in each dose group compared with NCG, and the liver glycogen in the low, middle and high dose groups was 1.56, 1.68 and 1.73 times that of the blank group, respectively, all of which were highly significant ($p < 0.01$). Similarly, muscle glycogen in the high-dose group was 1.98 times higher than NCG ($p < 0.01$). The results indicated that SCP could improve the reserve capacity of LG and MG in mice, and improve the energy metabolic system of mice, enhance the exercise endurance of the organism and delay the generation of fatigue.

3.2.4 SCP decreased BUN, LA in the blood

Protein decomposition produces amino acids, which are deaminated to produce NH_3 and CO_2 , both of which are synthesized into urea in the liver and then excreted through the kidneys. When the body exercises for a long time, the urea level will rise significantly and will be harmful to the body[26]. The effect of SCP on serum urea nitrogen in mice is shown in Fig. 3. The BUN levels in the SCP group were significantly lower than those in the NCG ($p < 0.01$), which indicated that SCP has the effect of promoting BUN decomposition and delaying protein metabolism.

Prolonged or high-intensity exercise increases oxygen consumption in muscles, causing hypoxia and accelerating glycolysis, which leads to lactate buildup when lactate production is greater than metabolism. A decrease in pH in muscle inhibits the activity of phosphofructokinase, which affects the rate of glycolytic synthesis of ATP, resulting in inadequate energy supply. In addition, acidification of body fluids affects the release and uptake of calcium ions, leading to muscle contraction disorders and ultimately to decreased exercise capacity, which is also a key cause of fatigue. As shown in Fig. 3, the LA content decreased in the SCP group compared with NCG ($p < 0.01$), indicating that SCP can reduce the lactate content in mice and has a certain anti-fatigue effect.

3.3 Effect of SCP on antioxidant enzyme system in mice

The generation and removal of reactive oxygen species (ROS) in living organisms is in a dynamic equilibrium, when various factors break this equilibrium and cause the concentration of reactive oxygen species to exceed the physiological limit and will damage biological macromolecules, antioxidant enzymes are the first line of defense against ROS. SOD is a very effective defense enzyme, and it plays an important role in antioxidant enzymes, it can remove free radicals in the body, but also remove free radical damage formed of "superoxide". As shown in Figure 4, the SCP group could increase the SOD

activity in the serum of mice, and there were significant differences between both the SCP and NCG groups ($p < 0.01$), indicating that SCP has the effect of scavenging free radicals in vivo. Malondialdehyde (MDA) is the end product of oxidative decomposition triggered by polyunsaturated fatty acid radicals, and therefore, it is a common biomarker of oxidative stress and one of the characteristic indicators of free radical metabolism[27], in addition, malondialdehyde polymers react with macromolecules such as proteins and deoxyribonucleic acid to mutate their structure, leading to impaired cellular function and cytotoxicity[28]. As shown in Figure 4, there was a decrease in MDA levels in the SCP group compared to NCG, all with significant differences ($p < 0.05$), indicating that SCP has an inhibitory effect on lipid peroxidation. CAT is the main H_2O_2 scavenging enzyme, which can catalyze the decomposition of H_2O_2 into H_2O and O_2 , prevent cell peroxidation and protect cells from free radical damage. As shown in Fig. 4, compared with NCG, both SCP groups can increase the CAT level in mice, and all of them are significant ($p < 0.05$), which indicates that SCP can improve the activity of CAT in vivo, accelerate the scavenging of reactive free radicals in vivo and protect cells. GSH-PX can reduce H_2O_2 or organic oxides to water and alcohol, and has the effect of protecting the structural and functional integrity of cell membranes as Fig. 4. Compared with NCG, both SCP can increase the activity of GSH-PX in mice serum, meanwhile, there is no significant difference in the low dose group ($p > 0.05$), and there is a significant difference in the middle and high dose group ($p < 0.01$). The results indicate that SCP has the effect of scavenging peroxides in mice.

It has been shown that strenuous exercise leads to increased production of free radicals and thus induces oxidative stress, which accelerates the production of free radicals or reactive oxygen species (ROS), which in turn damages cell membranes and other cellular components. SCP can significantly increase GSH-Px and SOD activity, reduce MDA content, actively scavenge ROS generated during exercise, inhibit the degree of lipid peroxidation, and effectively eliminate the harm caused by oxidative damage in mice after exhaustive exercise.

3.4 Histopathology

Skeletal muscle plays an important role in the regulation of the body's metabolism and exercise endurance. Morphological characteristics of muscle fibers, such as cross-section, are key indicators of the health and function of the musculoskeletal system, and excess free radicals during high-intensity exercise can cause changes in muscle structure. As shown in Fig. 5(a), the NCG has obvious muscle cells that were loosely arranged, appeared wrinkled, or had gaps between cells, as well as aggregation of skeletal muscle cell nuclei and necrosis and degradation of muscle fibers. While the PCG muscles showed significant contraction, the SCP group all showed varying degrees of improvement.

As shown in Fig. 5(b), myocardial fibers in the NCG were disorganized with wide gaps, and there were damaged cardiomyocytes and inflammatory cell infiltration. Compared with NCG, inflammatory cell infiltration was significantly improved or reduced in each treatment group.

As shown in Fig.5(c), the hepatocyte plates of NCG mice were irregularly arranged with uneven cytoplasmic staining. The cytoplasm was fused and swollen, and the nucleus was contracted. In addition, connective tissue proliferated along the periphery of blood vessels and inflammatory cells accumulated in mounds. Histopathological changes in the liver were improved to varying degrees in all treatment groups.

3.5 Pearson correlation analysis among different indicators

As shown in Figure 6, Pearson correlation analysis was used to analyze the correlation between different indicators. The changes of biochemical indicators in the body, such as the content of liver glycogen, muscle glycogen, lactate, and urea nitrogen can reflect the fatigue status of the body and evaluate the anti-fatigue activity. The results showed that liver glycogen, muscle glycogen, and LA, BUN were highly correlated. The antioxidant enzyme activity and MDA content were also highly correlated with LA and BUN. This indicates that there is a good correlation between the in vivo anti-fatigue effect and antioxidant activity of SCP.

It can be seen that SCP can effectively increase the activity of antioxidant enzyme system in mice, scavenge peroxides in cells, accelerate the scavenging of free radicals in the body, enhance the antioxidant defense mechanism, and then achieve the effect of relieving fatigue.

4. Discussion

In this study, the SCP of *Schisandra chinensis* polysaccharide was prepared by the traditional water extraction and alcoholic precipitation method, and the structure of SCP was characterized using Fourier transform infrared spectroscopy and ultraviolet spectroscopy. Then the effects of SCP on exercise endurance and its anti-fatigue effects in mice were evaluated in terms of both in vivo antioxidant pathways and fatigue-related physiological and biochemical indices (Fig. 7). The results showed that 1) SCP could prolong the weight-bearing swimming time and increase the content of liver glycogen and muscle glycogen; 2) SCP could reduce the accumulation of lactic acid and urea nitrogen in the blood, thus delaying the onset of fatigue or accelerating the recovery from fatigue; 3) SCP has strong antioxidant capacity, which could significantly increase the activity of antioxidant enzymes and reduce the content of MDA in mice; 4) SCP had protective effects on skeletal muscle, heart and liver of mice. This study showed that SCP has anti-fatigue function, which can prolong the exercise time and promote the recovery of fatigued body. It is expected that SCP will become a new natural and effective anti-fatigue product in the near future.

Related studies have shown that although glucose is a reducing monosaccharide, the amount of its content is not related to the antioxidant effect. Among all monosaccharide components, the content of rhamnose, arabinose, and galactose is closely related to the antioxidant effect. In addition, it was reported that polysaccharides containing a certain amount of galacturonic acid had a higher antioxidant capacity.

It has been pointed out that the antiviral activity of the individual components obtained by further purification and isolation of the refined polysaccharides was instead lower than that of the refined polysaccharides, suggesting that there may be a synergistic effect of the individual components of the polysaccharides. Some studies have shown that the antioxidant effect of *Ganoderma lucidum* polysaccharides is positively correlated with the content of its bound polyphenols, and protein content [23], while *Schisandra chinensis* polysaccharides are often combined with protein and polyphenols to form glycoproteins or other binders. Whether the antioxidant effect of *Schisandra chinensis* polysaccharides in vivo is synergistic or antagonistic with other components remains to be further investigated. Based on this observation, in order to fully investigate the interactions between SCP and other components, SCP can be further isolated and purified in future experiments, and then in vivo antioxidant studies and experiments in other directions can be conducted to identify the components that play specific roles, form a more in-depth characterization, and provide a theoretical basis for the deeper development of *Schisandra chinensis*.

Declarations

Author Contributions

Zhou Si wrote the main manuscript text, Haoxiang Chen, Chensi Gu, and Tingting Wang prepared the iconography and translation, and Ziluan Fan was the funder and manuscript reviewer and editor.

Ethics Approval Not applicable.

Consent for Publication

The present paper, which is original, has not been published before and is not currently being considered for publication elsewhere.

Consent to Participate

The present paper has been approved by all named authors.

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Data Availability

All data generated or analyzed for this study are included in this published article and will be made readily available upon request

Declaration of competing interest

The authors declare that they have no conflicts of interest.

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Figures

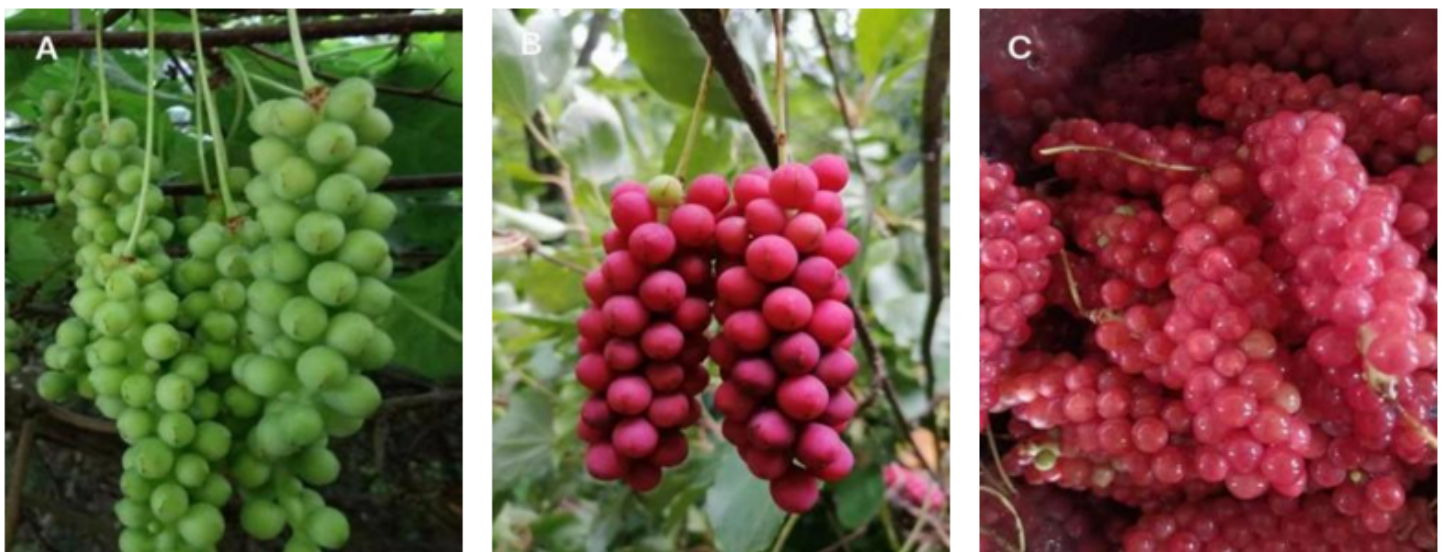


Figure 1

The above pictures describe the fruits of schisandra. green schisandra (A). ripe schisandra (B,C). All photos were taken by Zhang Yubao, in Shaanxi Province, China.

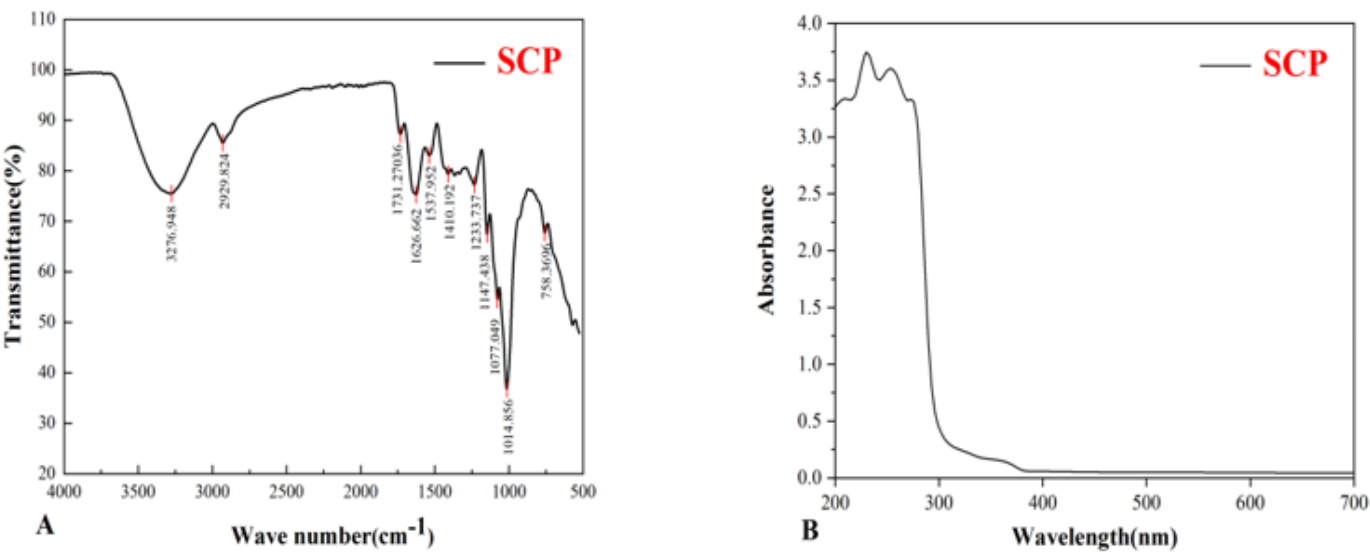


Figure 2

IR, UV profile of SCP.

A: IR spectrum of SCP; B: UV spectrum of SCP

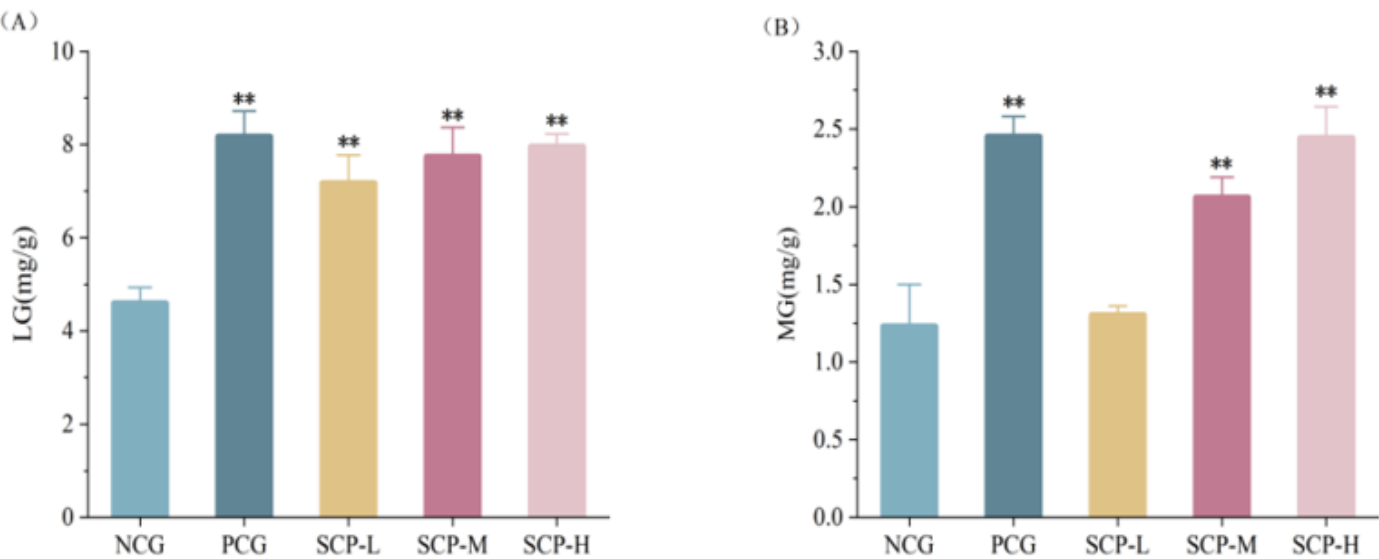


Figure 3

Effect of SCP on BUN (A) and LA (B) in mice.*P < 0.05, **P < 0.01 vs. negative control group.(n=8)

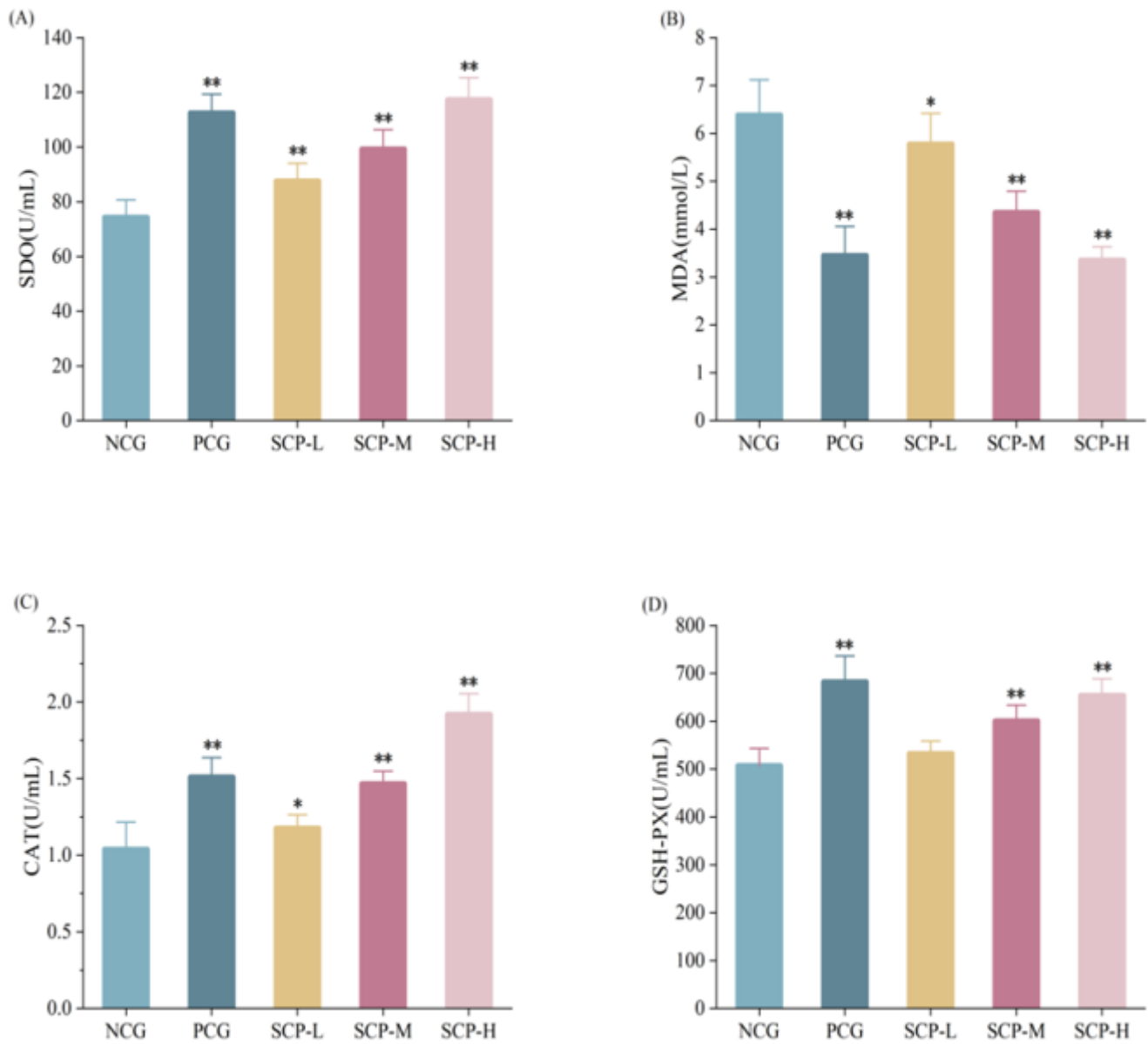


Figure 4

Effect of SCP on SOD (A), MDA(B), LA (C), and GSH-PX (D) in mice.*P < 0.05, **P < 0.01 vs. negative control group.(n=8)

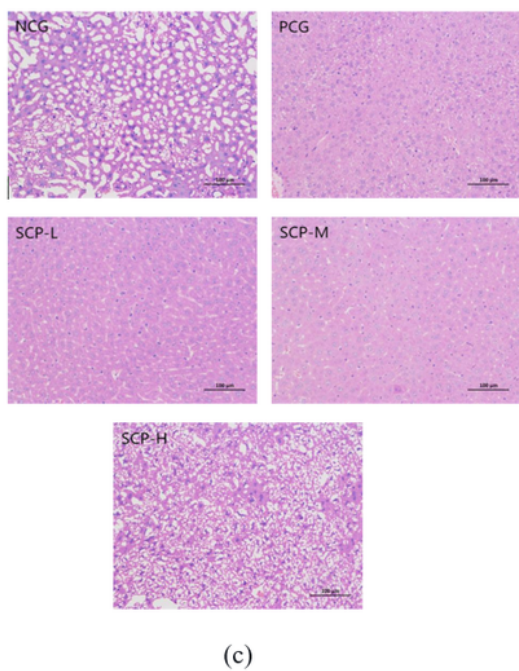
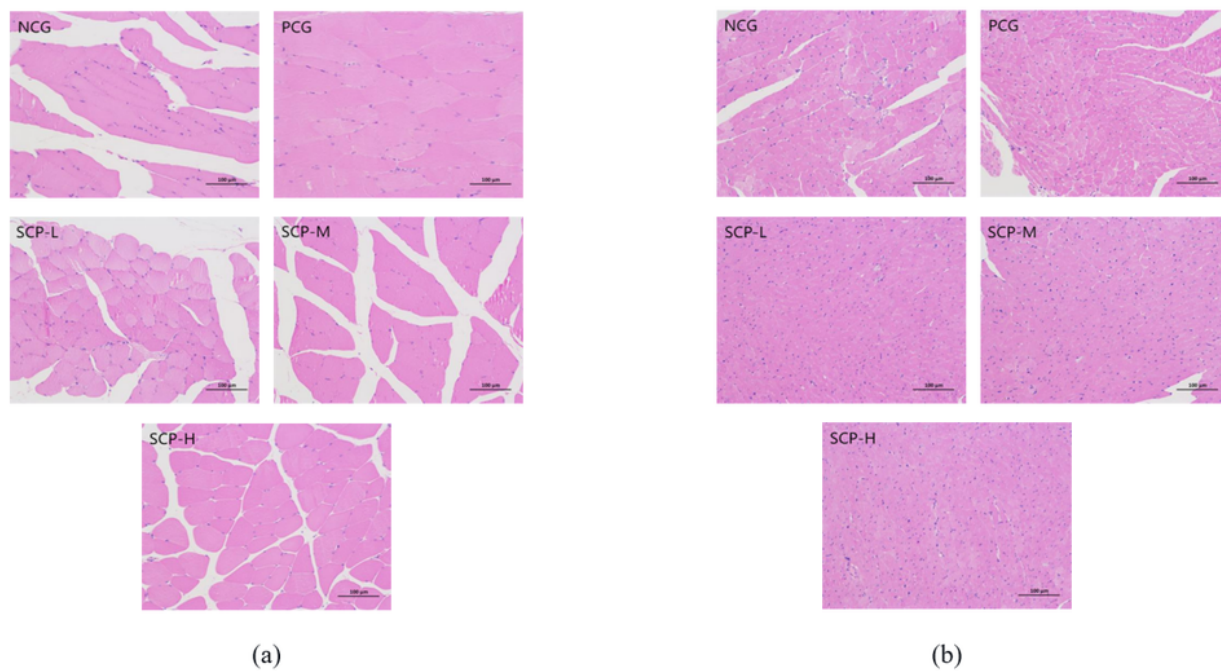


Figure 5

(a) H&E staining of skeletal muscle. (b) H&E staining of Heart. (c) H&E staining of liver tissues.

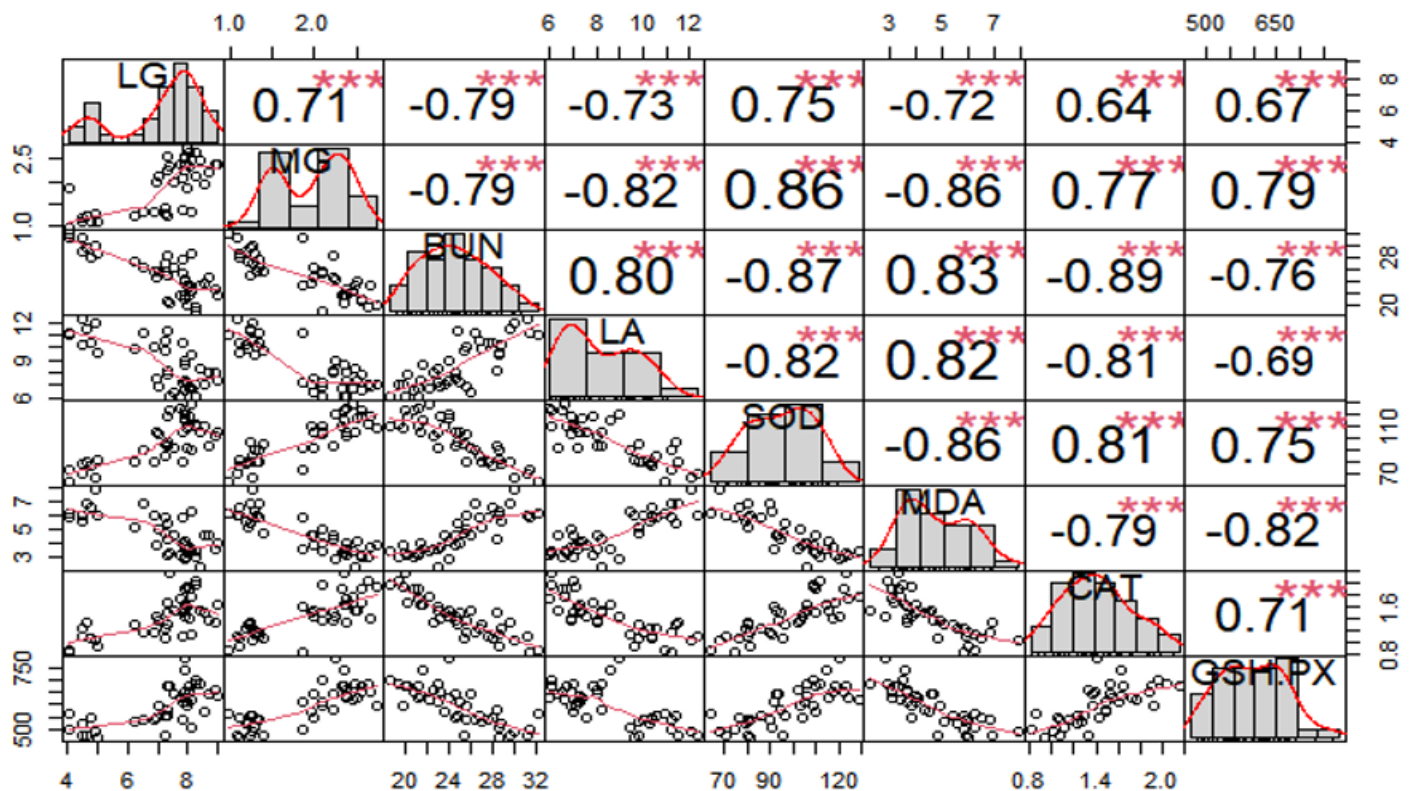


Figure 6

Pearson Correlation Analysis among different indicators. LG, liver glycogen; MG, muscle glycogen; BUN, blood urea nitrogen; LA, lactic acid; SOD, superoxide dismutase; MDA, malondialdehyde; CAT, catalase; GSH-Px, glutathione peroxidase. Significant correlations are indicated by * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$).

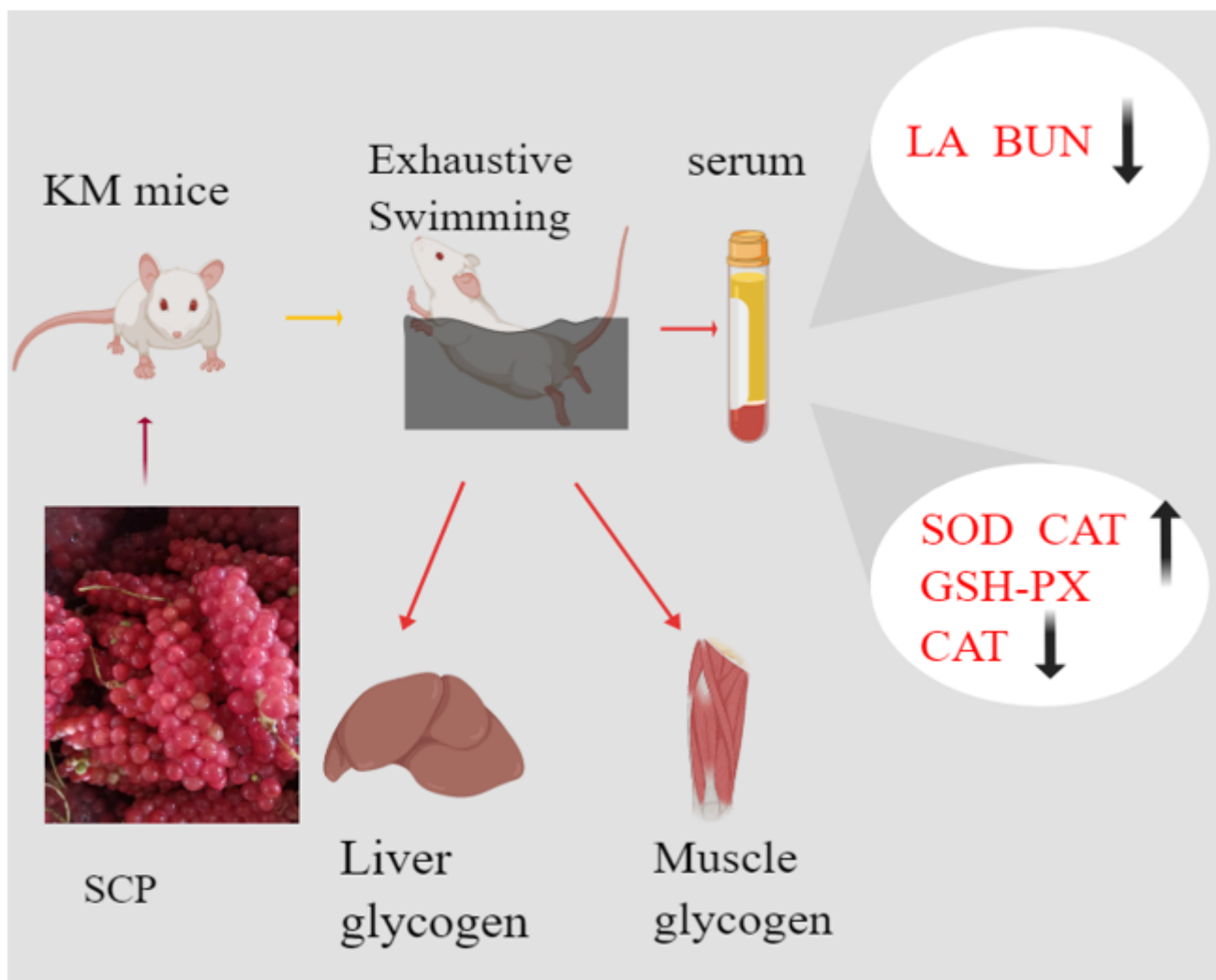


Figure 7

The tentative mechanism of anti-fatigue activities in vivo of SCP.