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Anti-fatigue Effects of Polysaccharide from *Angelica Sinensis*

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Abstract. The present study was designed to investigate the anti-fatigue effects of polysaccharides from *A. sinensis* (APS). The mice were divided into 4 groups: normal control, low, middle, and high-dose APS treated group. Normal control group was treated with distilled water, while APS treated groups received different doses of APS by oral gavage once daily for 28 day. The anti-fatigue effects were evaluated using the forced swimming test, along with the determination of exhaustive swimming times and fatigue related biochemical parameters such as blood lactic acid, serum urea nitrogen, liver glycogen, muscle glycogen, and serum creatine kinase. The data showed that APS could prolong the exhaustive swimming times in mice, reduce blood lactic acid production or promote blood lactic acid elimination, as well as decrease the serum urea nitrogen contents, and increase contents of liver and muscle glycogen. Therefore, APS has anti-fatigue effects.

1. Introduction

It's difficult to give the definition of fatigue, which can generally be understood as the difficulty of starting or maintaining voluntary activities. At the 5th International Biochemistry Conference in 1982, fatigue was defined as "the physiological processes of the body when it cannot continue its function to a certain extent or maintain a predetermined exercise intensity" [1]. Fatigue is a physiological protection phenomenon that is inevitable when the human brain or physical activity reaches a certain level. It can be divided into two categories: physical fatigue caused by factors such as forced exercise or sustained heavy physical labor, and mental fatigue caused by sleep deprivation, etc. Physical fatigue is the body experience of physical exertion after exercise, and mental fatigue is subjective self-reported weariness. As the treatment methods and drugs for fatigue in modern medicine are limited, and because chemical synthesis drugs have strong side effects, their application has been greatly limited[2]. In the search for safer and more effective anti-fatigue methods and drugs, antifatigue compounds derived from natural products have become hot spots in the study because of their few side effects and their lack of harmful components on the body.

Dong quai is the root of *Angelica sinensis* (Oliv.) Diels, which is a well-known traditional Chinese medicine and has the effects of activating blood circulation and regulating the circulation of the intestines [3]. Polysaccharides is one of the main components of *A. sinensis*. In recent years, there has been much progress in the study of the constituents and pharmacology of polysaccharides from *A. sinensis* (APS), and the monosaccharides of APS consist of rhamnose, arabinose, mannose, glucose and galactose. APS have a wide range of biological and pharmacological properties, such as hematopoiesis, immunomodulatory, antitumor, antioxidant, radioprotection, hypoglycemic, hepatoprotective effects, and so on [5,6]. However, the current literature on anti-fatigue effects of APS

is rarely reported. Therefore, the present study investigated the anti-fatigue effects of APS. This will provide scientific basis for the further development and utilization of APS in sports nutrition field.

2. Materials and methods

2.1. Plant materials

The dried Dong quai (the root of *A. sinensis*) were purchased from a local pharmacy and authenticated by Dr. Liu J.H (School of Life Sciences, Hunan Normal University, Changsha, China).

2.2. Chemicals and reagents

Glucose standards were purchased from Dalian Meilun Biotechnology Co., Ltd. (Dalian, China). The assay kits for the determination of lactic acid, urea nitrogen, liver glycogen, and muscle glycogen were purchased from Nanjing Jiancheng Company (Nanjing, China); The assay kit for the determination of creatine kinase (CK) was purchased from Zhongsheng Reagent Company (Beijing, China).

2.3. Preparation of the polysaccharides from A. sinensis (APS)

The dried Dong quai was crushed by an electric grinder and then sieved (60 mesh). The powder sample (200 g) was defatted twice with chloroform and methanol solution (2:1, v/v) at 60 °C for 4 h and the residue was extracted thrice with distilled water at 90 °C for 3 h. The filtrates were combined and concentrated to 30 mL in a rotary evaporator and deproteinated using the Sevag method. The concentrated extract was precipitated with five volumes of 95% ethanol at 4°C for 12 h, then the precipitate was collected by centrifugation (2000 r/min for 5 min), and washed sequentially with 95% ethanol, absolute ethanol and acetone, and finally vacuum dried to obtain polysaccharides from *A. sinensis* (APS). The polysaccharides content was determined by phenol-sulfuric acid method and the extraction yield of APS (polysaccharide mass / Dong quai sample mass) was 5.64%.

2.4. Experimental animals

Healthy male Kunming mice (weighing 20 ± 2 g) were purchased from Hunan biological supplier (Changsha, China). Animals were reared in standardized animal room and fed with formulated rodent chow. The composition of rodent chow was 20% corn starch, 16% rice, 19% bran, 20% soybean oil, 16%fishmeal, 3% calcium powder, 3% bone meal, 2.3% yeast powder, 0.5% salt, 0.1% vitamin, and 0.1% minerals. The animal room temperature was 23 ± 2 °C, the humidity was 55 ± 5%, and 12 h light/dark cycle. During the experimental period, the mice were allowed free access to rodent chow and tap water. All animal handling procedures were performed in strict accordance with the Guideline on the Humane Treatment of Laboratory Animals (MOST 2006) and approved by the Animal Ethics Committee of Central South University (Changsha, China).

2.5. Experimental design

The mice were acclimated to the laboratory environment for one week and then used in experiments. The mice were randomly divided into 4 groups of 16 animals each according to their body weight. The first group was served as normal control (NC) group, the second group was served as low-dose APS treated (50 mg/kg.d, LAT) group, the third group was served as medium-dose APS treated (100 mg/kg.d, MAT) group, and the fourth group was served as high-dose APS treated (200 mg/kg.d, HAT) group. APS was dissolved in 1.0 mL of distilled water, and the control group was treated with the same volume of distilled water. The treatments were administered orally by gavage once daily for 28 days.

After 28 days, 8 mice were taken out from each group for the forced swimming test. The procedure was modified from previously described [7]. Briefly, 30 min after the last treatment, the mice were placed in a swimming pool (50, 40, and 40 cm in length, width, and height, respectively) to swim until exhaustion. The tail root of each mouse was loaded with a lead block, which weighed equal to 5% of its body weight. The mice were assessed to be exhausted when theirs head sank into the water and

they were not able to return spontaneously to the surface within 10 s period, and the exhaustive swimming times were recorded. Then, the mice were immediately taken out, and the blood samples were collected by rapidly removing the eyeball under ether anesthesia. Serum was prepared by centrifugation at 3000 r/min for 15 min, and was used for the determination of contents of urea nitrogen, and creatine kinase (CK) according to the instructions of kits. The mice were sacrificed after blood collection, the liver and quadriceps were harvested and rinsed with 0.9% physiological saline, and blotted dry with filter paper. The contents of liver and muscle glycogen were measured according to the instructions of kits.

After 28 days, the remaining 8 mice were taken out from each group, and forced to swim in the swimming pool (weight-unloaded) for 30 min. Before and after swimming, blood samples were collected from orbital sinus with a capillary tube, and the blood lactic acid contents were measured according to the instructions of kit.

2.6. Data analysis

The data were expressed as mean ± standard deviation (SD). Data analyses were performed by analysis of variance (ANOVA) and t-test, and p values <0.05 were considered statistically significant.

3. Results and discussion

3.1. Effect of APS on the exhaustive swimming times of mice

It is well documented that the most important physiological effect of fatigue is the energy metabolism of muscle activity, and exercise endurance is an important factor for evaluating anti-fatigue treatment [8]. Forced swimming test is an experimental animal model for evaluating the exercise endurance, which could cause blood biochemical changes and has high reproducibility [9].

From Figure 1, it can be seen that the exhaustive swimming times in the APS treated (LAT, MAT, and HAT) groups were significantly higher than that of the NC group (p<0.05). These results show that APS could enhance exercise endurance and delay physical fatigue, and the effect of APS is dose-dependent.

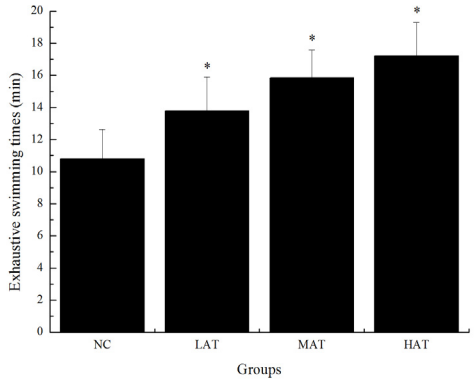

Figure 1. Effect of APS on the exhaustive swimming times of mice. Data were expressed as mean ± standard deviation (SD), *p<0.05 compared with normal control (NC) group.

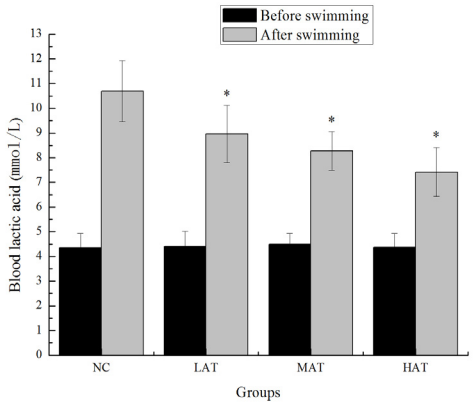

Figure 2. Effect of APS on the blood lactic acid of mice. Data were expressed as mean ± standard deviation (SD), *p<0.05 compared with normal control (NC) group.

3.2. Effect of APS on the blood lactic acid of mice

Lactic acid is the product of glycolysis under anaerobic conditions. In strenuous exercise, muscle must obtain enough energy by anaerobic glycolysis and produce large amounts of lactic acid through

glycolysis. The conversion of lactic acid to glucose through gluconeogenesis is one of the main ways to remove excess lactic acid from strenuous exercise, while excess glucose will be preserved as liver glycogen. In addition, lactic acid can also be used to supplement muscle glycogen storage [10]. Increased lactic acid could lower pH, resulting in a variety of physiological and biochemical side effects, including glycolysis, phosphofructokinase, and calcium ion release caused by muscle contractione, which were harmful to the body performance. [11]. Blood lactic acid is a sensitive index of fatigue status, and rapid elimination of lactic acid or reduction of lactic acid accumulation would help relieve fatigue.

From Figure 2, it can be seen that blood lactic acid contents were not significantly different between APS treated (LAT, MAT, and HAT) groups and the NC group before swimming ($p>0.05$). After swimming, the blood lactic acid contents in the APS treated (LAT, MAT, and HAT) groups were significantly lower than that of the NC group ($p<0.05$). These results show that APS could reduce blood lactic acid production or promote blood lactic acid elimination during exercise to delay physical fatigue

3.3. Effect of APS on the serum urea nitrogen of mice

Urea nitrogen is the main catabolic product of the body's proteins. Protein is less catabolic metabolism when the body's exercise is less. After strenuous exercise, proteins make catabolism stronger to compensate for energy shortage caused by catabolism of carbohydrate and fat, resulting in increased urea nitrogen content. The more urine nitrogen is produced when the physical load is greater [12]. Urea nitrogen is closely related to exercise endurance, body function and fatigue. Therefore, urea nitrogen is one of the indexes reflecting the degree of fatigue.

From Figure 3, it can be seen that the serum urea nitrogen contents in the APS treated (LAT, MAT, and HAT) groups were significantly lower than that of the NC group ($p<0.05$). These results show that APS could reduce the degree of protein breakdown to provide energy, so as to relieve physical fatigue by saving protein effect.

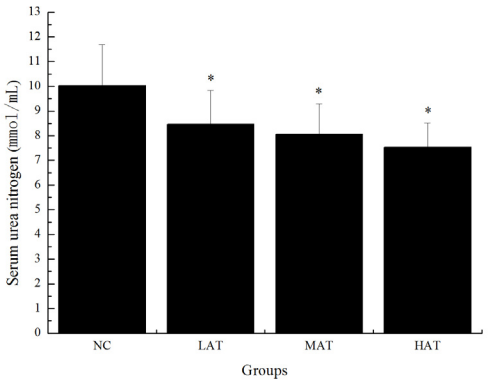

Figure 3. Effect of APS on the serum urea nitrogen of mice. Data were expressed as mean ± standard deviation (SD), *$p<0.05$ compared with normal control (NC) group.

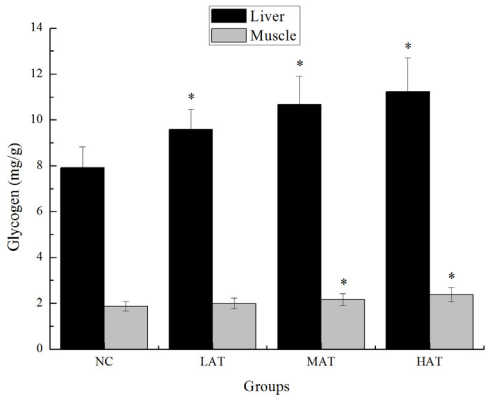

Figure 4. Effect of APS on the liver and muscle glycogen of mice. Data were expressed as mean ± standard deviation (SD), *$p<0.05$ compared with normal control (NC) group.

3.4. Effect of APS on the liver and muscle glycogen of mice

Energy originally originates from the breakdown of muscle glycogen during strenuous exercise, at a later stage, energy comes from liver glycogen. Glycogen can supplement the glucose consumption during exercise to maintain the stability of glucose in the physiological range [13]. The glycogen

reserve is of great significance for the improvement of exercise endurance. The rapid depletion of liver and muscle glycogen is one of the important factors to accelerate the appearance of physical fatigue.

From Figure 4, it can be seen that the liver glycogen contents in the APS treated (LAT, MAT, and HAT) groups, and muscle glycogen contents in the medium-dose and high-dose APS treated (MAT, and HAT) groups, were significantly higher than that of the NC group ($p<0.05$). Although low-dose APS treated (LAT) group also had a small increase in muscle glycogen, but there was no statistical significance ($p>0.05$). These results show that APS could delay physical fatigue probably caused by increasing glycogen reserve or reducing the glycogen consumption to maintain blood glucose balance in the body, providing energy for the organization.

3.5. Effect of APS on the serum creatine kinase of mice

Creatine kinase (CK) is an important metabolic enzyme in muscle. The normal function of CK in cells is to add a phosphate group to sarcosine and become a high-energy macromolecular substance, creatine phosphate, which could rapidly burn to generate energy to supply cells. Most of the CK in the body is present in muscles. When muscles are damaged, muscle cells break down and CK enters the blood. Therefore, an increase in blood CK content indicates that muscle damage has occurred or is occurring [14].

As can be seen from Figure 5, compared with the NC group, serum CK levels in the in the APS treated (LAT, MAT, and HAT) groups were slightly reduced, but there was no statistical significance ($p>0.05$). These results show that APS has no excessive protection against exercise-induced muscle damage.

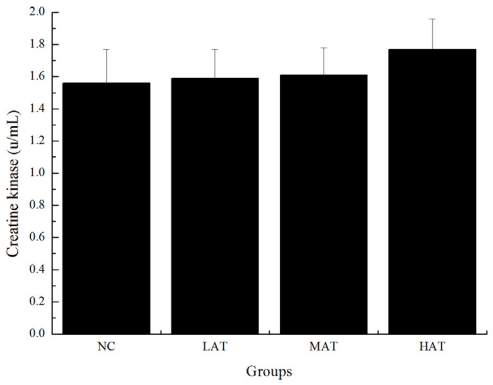

Figure 5. Effect of APS on the serum creatine kinase of mice. Data were expressed as mean ± standard deviation (SD), *$p<0.05$ compared with normal control (NC) group.

4. Conclusions

The present findings showed that APS has anti-fatigue effects, which could prolong the exhaustive swimming times in mice, reduce blood lactic acid production or promote blood lactic acid elimination, as well as decrease the serum urea nitrogen contents, and increase contents of liver and muscle glycogen. However, further studies are needed to elucidate the specific mechanisms of anti-fatigue effects of APS.

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