Effects of polysaccharide from *Angelica sinensis* on some related indexes of free radical and energy metabolism after strenuous exercise in rats

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**Abstract.** The root of *Angelica sinensis* is a well-known Traditional Chinese Medicine. Present study was designed to evaluate the effect of polysaccharides from *Angelica sinensis* (APS) on the some related indexes of free radicals and energy metabolism after strenuous exercise. The animals were divided into three groups, namely the sedentary control (SC), exercise control (EC), and exercise APS treated (200 mg/kg.d, EAT) group. At the 28th day, the rats in the EC and EAT group performed three-stage incremental exercise until exhaustion. After exhaustion exercise, a series of biochemical parameters including blood glucose, superoxide dismutase (SOD), glutathione peroxidase (GPx), malondialdehyde (MDA) and free fatty acid (FFA) were measured. The results showed that APS increased exhaustion times, blood glucose levels, FFA levels in plasma and the levels of SOD and GPx in liver, which were accompanied by corresponding decreases in the MDA levels in liver. The present findings indicate that the anti-fatigue mechanisms of APS may be attributed to the following aspects: 1) APS can enhance antioxidant enzymes activities and inhibit the production of lipid peroxidation products, which can protect the body from oxidative damage caused by free radicals. 2) APS can affect carbohydrates metabolism, which has the effects of increasing hepatic and muscle glycogen stores and maintaining homeostasis of blood glucose levels. 3) APS can affect fat metabolism, which can regulate the formation of FFA and the utilization of fat oxidation. APS can be used as a nutritional supplement in exercise training.

1. Introduction

*Angelica sinensis* (Oliv.) Diels (*A. sinensis*) is a perennial herb that is mainly distributed in Gansu and Yunnan area in China. Dong quai is the root of *A. sinensis*, which is a well-known Traditional Chinese Medicine and has the effects of activating blood circulation, adjusting menstruation and relieving pain, and regulating the circulation of the intestines [1]. Several studies have indicated that *A. sinensis* contains a variety of chemical components, including volatile oils, organic acids, carbohydrates, coumarins, flavonoids, amino acids, trace elements and so on [2]. The carbohydrates contained in *A. sinensis* are mainly glucose, fructose, sucrose (40%) and polysaccharide (8%) [3]. In the past decades, it was believed that the main active components of *A. sinensis* were volatile oil and ferulic acid, but with the in-depth study of polysaccharides from *A. sinensis* (APS), the biological activity of APS has attracted great attention from researchers. The monosaccharides of APS are composed of glucose, galactose, xylose, arabinose, mannose, rhamnose, glucuronic acid and galacturonic acid [4]. APS obtained by different separation methods have slightly different monosaccharide types. Recent pharmacological studies have demonstrated that APS have many biological activities, such as anti-
oxidation, immune regulation, anti-tumor, anti-radiation damage, anti-virus, anti-aging and liver protection [5].

Exercise-induced fatigue is an extremely complex process of comprehensive changes in body. Decreased muscle performance and function is the basic sign and essential characteristics of exercise-induced fatigue. The fatigue experienced during exercise is distinct from the general fatigue experienced by the estimated 7-45 % of the population, because of its transient and replicable nature [6]. At the Fifth International Sports Biochemistry Conference (1982), exercise-induced fatigue was defined as: "The physiological process of the body cannot continue its function at a certain level or cannot maintain the predetermined exercise intensity" [7]. Exhaustion refers to continuing to exercise while fatigue, until the muscles or organs can not maintain exercise, which is a special form of fatigue. With the improvement of the level of modern competitive sports, the intensity of exercise is gradually increasing, which leads to the increasing attention of the recovery of fatigue. Our previous studies have confirmed the anti-fatigue effects of APS [8], but its mechanism is not clear. This study was designed to investigate the effect of APS on the some related indexes of free radicals and energy metabolism after strenuous exercise using a forced animal running test, which will explore the anti-fatigue mechanisms of APS from antioxidant system and energy metabolism system. The results of this study will also provide theoretical guidance and experimental basis for the application of APS as a nutritional supplement in exercise training.

2. Materials and methods

2.1. Plant materials and chemicals
The dried root of *A. sinensis* was purchased from a local pharmacy (Changsha, China). The assay kits for the determination of superoxide dismutase (SOD), glutathione peroxidase (GPx), malondialdehyde (MDA), glycogen, and free fatty acid (FFA) were purchased from Nanjing Jiancheng Company (Nanjing, China). All the other chemicals used in this study were obtained from the local supplier (Changsha, China).

2.2. Instruments and equipment
Blood glucose meter (Sannuo Biosensor Co., Ltd., China), AU680 automatic biochemical analyzer (Beckman Coulter, USA), Multiskan FC microplate reader (Thermo Fisher Scientific, USA), TGL20MW desktop high-speed cryogenic centrifuge (Xiangyi Centrifuge Instrument Co., Ltd., China), F20 grinder (Shenzhen Leitong Industrial Co., Ltd., China), RE52CS rotary evaporator (Shanghai Rongsheng Instrument Factory, China), SHB-III circulating water multi-purpose vacuum pump (Zhengzhou Changcheng Branch Industry and Trade Co., Ltd., China).

2.3. Animals and breeding conditions
6-8 weeks male Sprague Dawley (SD) rats, weighing 180-200 g, were provided by Hunan biological supplier (Changsha, China) and acclimatized for one week before being used. Animals were reared in standardized animal room (12-hour light/12-hour dark cycle, 23 ± 2 °C, 55 ± 5% humidity) and fed with formulated rodent chow. During the experimental period, the rats were allowed free access to 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).

2.4. Preparation of the polysaccharides from *A. sinensis* (APS)
The APS were prepared according to previously method [9]. Briefly, the dried powder sample was defatted twice with chloroform and methanol solution 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, concentrated 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, and washed sequentially with 95% ethanol, absolute ethanol and acetone, and finally vacuum dried to obtain APS.

2.5. Animals grouping and treating
The rats were randomly divided into three groups of ten animals each according to their body weight. The first group was served as sedentary control (SC) group, the second group was served as exercise control (EC) group, and the third group was served as exercise APS treated (200 mg/kg.d, EAT) 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 21 days, the rats in the EC and EAT groups were running exercise on a motor-driven treadmill (SA101, Jiangsu Sai Ansi Biotechnology Co., Ltd., China). The speed, duration and, slope of each exercise session were constant at 10 m/min, 10 min, and 0° for a week, respectively to accustom the rats to running exercise. At the 28th day, the rats in the EC and EAT groups performed three-stage incremental exercise until exhaustion (Figure 1). The first phase of exercise: the speed, duration and, slope of exercise at 10 m/min, 10 min, and 0°, respectively. The second phase of exercise: the speed, duration and slope of exercise at 20 m/min, 15 min, and 5°, respectively. The third stage of exercise: the speed and slope of exercise at 30 m/min and 10°. When the rats stopped running, they will be immediately shocked by the platform power until exhausted, meanwhile exhaustion times were recorded. After exhaustion, the blood were collected via abdominal aorta puncture under ether anesthesia, and the rats were sacrificed by decapitation. The blood is placed in an anticoagulant tube, and the plasma was prepared by centrifugation at 2000 × g for 15 min at 4 °C. Liver was harvested and rinsed with 0.9% physiological saline, blotted dry with filter paper, weighed and frozen immediately in liquid nitrogen and stored at -80°C for future analysis. A series of biochemical parameters including blood glucose, SOD, GPx, MDA and FFA were determined according to the instructions of kits.

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. Effects of APS on the exhaustion running times of rats
The laboratory rat is a commonly used animal model for investigating exhaustive exercise effects on biochemical changes in humans [10]. The effects of APS on the exhaustion running times of rats are shown in Figure 2. Compared with the EC group, the exhaustion running times in the EAT group were significantly longer (p<0.05). These results once again indicated that APS has anti-fatigue effects.

3.2. Effects of APS on the SOD, GPx and MDA in liver of rats
Under normal physiological conditions, the formation and elimination of free radicals in the body maintains a dynamic balance. Numerous studies have proved that strenuous exercise can produce a large amount of free radicals, exceeding the body's ability to scavenge free radicals, which can cause
oxidative stress [11]. There is a linear relationship between the increase of free radicals and the decrease of exercise endurance, which is mainly manifested by the lipid peroxidation in various biofilms of the body caused by free radicals. GPx and SOD are two key antioxidant enzymes that eliminate free radicals. SOD can catalyze the disproportionation of superoxide radicals into oxygen and hydrogen peroxide. GPX can reduce hydrogen peroxide or organic hydroperoxide to water and alcohol, respectively [12]. MDA is a lipid peroxidation product formed by free radicals attacking polyunsaturated fatty acids in biofilms, which is a good marker for measuring the degree of free radical damage in the body [13]. The effects of APS on the SOD, GPx and MDA in liver of rats are shown in Figure 3. Compared with the SC group, the levels of SOD and GPx in the EC and EAT groups were significantly lower (p<0.05), and the MDA level in the EC and EAT groups was significantly higher (p<0.05). Compared with the EC group, the levels of SOD and GPx in the EAT group were significantly higher (p<0.05), and the MDA level in the EAT group was significantly lower (p<0.05). These results indicated that APS can enhance antioxidant enzymes activities and inhibit the production of lipid peroxidation products.

3.3. Effects of APS on the blood glucose of rats

Energy for exercise is originally derived from the decomposition of glycogen. During strenuous exercise, the body preferentially uses muscle glycogen for energy supply. After a large amount of muscle glycogen is consumed, the body decomposes the hepatic glycogen to release glucose to maintain homeostasis of blood glucose levels [14]. As the exercise time prolongs, hepatic glycogen is consumed in large quantities. At this time, the amount of glucose released into the blood by the decomposition of hepatic glycogen is greatly reduced, and the skeletal muscle still takes a large amount of glucose from the outside of the cell to meet the energy requirement of muscle contraction, thereby causing hypoglycemia [15]. Decreased blood glucose can lead to insufficient energy supply in the central nervous system and decreased exercise capacity, which leads to fatigue. Our previous studies have confirmed that APS can increase hepatic and muscle glycogen stores during strenuous exercise [8]. Effects of APS on the blood glucose of rats are shown in Figure 4. Compared with the SC group, the blood glucose levels in the EC and EAT groups were significantly lower (p<0.05). Compared with the EC group, the blood glucose levels in the EAT group were significantly higher (p<0.05). These results indicated that APS has the effects of increasing hepatic and muscle glycogen stores and maintaining homeostasis of blood glucose levels. This effect of APS can ensure the energy
supply of the central nervous system, muscles and red blood cells, delay the occurrence of fatigue, which may be one of its anti-fatigue mechanisms.

3.4. Effects of APS on the FFA in plasma of rats
Fat is an important source of energy in the body. It is well known that after long-term strenuous exercise, the glycogen is depleted, and the fat metabolism is strengthened, so that the free fatty acid (FFA) in the plasma is increased [16]. The body uses the increased FFA for oxidative metabolism, which is beneficial to improve exercise endurance. However, when the fat is over-mobilized, the production rate of FFA in plasma exceeds the fat oxidation utilization rate, causing a significant increase in FFA, which will weaken the hydrolysis of ATPase by sodium-activated adenosine triphosphatase (Na^+-ATPase) and calcium-activated adenosine triphosphatase (Ca^{2+}-ATPase). This function hinders the formation of action potentials in the muscle cell membrane and the absorption of Ca^{2+} by the sarcoplasmic reticulum, affecting the contraction and relaxation of the muscles, thereby causing fatigue [17]. In addition, since FFA in plasma can compete with tryptophan for binding to albumin, which leads to an increase in free tryptophan levels in plasma. Tryptophan is a precursor of serotonin, and elevated levels of serotonin can also cause fatigue in the central nervous system [18].

Effects of APS on the FFA in plasma of rats are shown in Figure 5. Compared with the SC group, the FFA levels in the EC and EAT groups were significantly higher (p<0.05). Compared with the EC group, the FFA levels in the EAT group were significantly lower (p<0.05). These results indicated that APS can regulate the formation of FFA and the utilization of fat oxidation, which may also be one of its anti-fatigue mechanisms.

4. Conclusions
The results of the study found that the anti-fatigue mechanisms of APS may be attributed to the following aspects: 1) APS can enhance antioxidant enzymes activities and inhibit the production of lipid peroxidation products, which can protect the body from oxidative damage caused by free radicals. 2) APS can affect carbohydrates metabolism, which has the effects of increasing hepatic and muscle glycogen stores and maintaining homeostasis of blood glucose levels. 3) APS can affect fat metabolism, which can regulate the formation of FFA and the utilization of fat oxidation. However,
further research to clarify its anti-fatigue molecular mechanisms is necessary for the practical application of APS.

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