

Full Length Research Paper

Effect of *Astragalus membranaceus* polysaccharides on oxidative damage in skeletal muscle of exhaustive exercise rats

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The aim of this study was to determine the effects of *Astragalus membranaceus* polysaccharides (AMP) on oxidative damage in skeletal muscle of exhaustive exercise rats. The animals were divided into four groups at random, with eight rats in each group. Group 1, assigned as a control group, received normal saline (vehicle); groups 2 to 4 received AMP at different dosages (50, 100 and 200 mg/kg bw) respectively for 30 successive days. Rats were made to perform an exhaustive running test on a treadmill at a final speed of 30 m/min, 10% grade, at approximately 70 to 75% VO₂max. The mean endurance times of running on a treadmill to exhaustion of each rat were examined. And skeletal muscle samples were collected for determining the activity of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT) and levels of malondialdehyde (MDA). The results showed groups 2 to 4 significantly increased in exhaustive exercise time compared with groups 1. MDA concentration of skeletal muscle of the groups 2 to 4 were significantly decreased compared with that of the groups 1. The antioxidant enzyme activities of SOD, GSH-Px and CAT of skeletal muscle of the groups 2 to 4 significantly increased compared with that of the groups 1. It is concluded that AMP supplementation increased performance of exhaustive exercise of rats. And it is beneficial for increasing antioxidant status and inhibits oxidative stress induced by exhaustive exercise.

Key words: *Astragalus membranaceus* polysaccharides, oxidative damage, exhaustive exercise, rats.

INTRODUCTION

During physical exercise, oxygen flux to active skeletal muscles increases, which leads to enhanced production of reactive oxygen species (ROS) and free radicals (Chiaradia et al., 1998). Production of ROS in quantities that overwhelm the endogenous antioxidant defense system has been referred to as oxidative stress (Bloomer et al., 2006; Goldfarb et al., 2007). Regular physical exercise has beneficial effects on health, and acute exhaustive exercise may attenuate these benefits via the induction of oxidative stress, which if increased can be harmful to all cellular macromolecules, such as lipids, proteins, and DNA (Niu et al., 2008). The supplementation

of exogenous antioxidants may provide support for the endogenous antioxidant defense system to assist in the handling of the increased production of ROS (Aguiló et al., 2005).

The roots of *Astragalus membranaceus* (AM) are one of the most popular health-promoting plants in East Asia, and have been used as herbs for more than 2000 years (Lee et al., 2007). The main constituents of AM roots are polysaccharides, saponins, flavonoids, amino acids, and trace elements (Zheng et al., 1998; Ma et al., 2002). Recently AMP is reported to have antioxidant, anti-diabetic, anti-hypertensive, and immunomodulatory activities (Wu et al., 2005; Bian and Li, 2009; Zhang et al., 2009; Yang et al., 2009), but few researches have been reported on the effects of AMP on exercise function. In this study, we investigated the effects of AMP on oxidative damage in skeletal muscle of exhaustive

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exercise rats.

MATERIALS AND METHODS

Reagents

SOD, GSH-Px, CAT and MDA assay kits were purchased from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China); Other chemicals were of analytical-reagent grade and were purchased from Kunming Chemical Reagent Co. (Yunnan, China), unless otherwise stated.

Experimental animals

Male inbred Sprague-Dawley strain rats, 6 to 8 weeks old, were raised under controlled environment in the institute's animal house at $25 \pm 1^\circ\text{C}$ and 12 h light-dark cycle. The animals were fed standard animal food, that is, pellet and water ad libitum. All the experiments were performed based on the regulations specified by the Institutional Animal Ethical Committee (IAEC) and conformed to national guidelines on the care and use of laboratory animals, China.

Plant materials and preparation of *A. membranaceus* polysaccharides

The roots of *A. membranaceus* (AM) were purchased from Kunming herb market (Yunnan, China), and identified by Professor Yang of Kunming University of Science and Technology. Voucher specimens (KMY-DK2073) were preserved in Yunnan Natural Product Research Institute. AMP was prepared by the method of Sun et al. (2005). The dried roots of AM were ground to fine powder, and then extracted with hot water (65°C) for three times (1:35, w/v). The extract was concentrated in a rotary evaporator under reduced pressure, precipitated by 95% (v/v) ethanol at 4°C for 24 h, and then centrifuged (10 min, 3000 rpm). The sediment was washed with ethanol, acetone, and ether alternately for three times to exclude lipid groups. The precipitate was vacuum freeze-dried, and crude AMP were obtained, which was dissolved in water and discolored by H_2O_2 , then proteins were removed by the sewage method (Zhang et al., 2006). Furthermore, this crude polysaccharide was suspended in water, dialyzed to eliminate small molecules to obtain the pure polysaccharide, which was collected and precipitated with 95% (v/v) ethanol and then lyophilized. The yield of AMP was 2.07%.

Exercise protocol

The animals were divided into four groups at random, with eight rats in each group. Group 1, assigned as a control group, received 1.5 ml of sterile normal saline (vehicle) for 30 successive days. While groups 2 to 4 received AMP at different dosages (50, 100 and 200 mg/kg bw) respectively for 30 successive days, The AMP were redissolved in 1.5 ml of sterile normal saline and administered orally by a canule.

Before exercise test, the rats were introduced to running on a treadmill with 15 to 20 min exercise bouts at 15 to 30 m/min for 7 days to accustom them to running (Ji et al., 1991). The treadmill was equipped with an electric shocking grid on the rear barrier to motivate the animal to run (PBC47700 treadmill device; Coulbourn Instruments Co., USA). On the day of the exercise test, they were required to run to exhaustion on a five-lane inclined (10°) treadmill at a final speed of 30 m/min, which was approximately 70 to 75% of

VO_2max (Brooks and White, 1978). The measurement of maximal O_2 consumption (100% VO_2 max) was considered valid only when the animal could no longer maintain pace with the treadmill (Somani et al., 1995). Therefore, exhaustion was determined as the rat being unable to upright itself when placed on its back. To eliminate diurnal effects, the experiments were performed at the same time (08.30 to 11.30 h).

Tissue preparation

After exhaustive exercise, rats were anaesthetized with thiopentone sodium (50 mg/kg) skeletal muscle was excised immediately and immersed in physiological saline. For the preparation of skeletal muscle homogenates (1 g of tissue plus 10 ml homogenization buffer), the frozen pieces were thawed on ice and then homogenized. The suspension was centrifuged at $671 \times g$ at 4°C for 10 min and clear supernatant was used for the following estimations of activity of SOD, GSH-Px, CAT and levels of MDA.

Assay of oxidation products and enzymatic antioxidants

Lipid peroxidation was assessed by determining the level of MDA in skeletal muscle homogenates by using commercial diagnostic kit. Enzymatic antioxidants were assessed by determining the activity of SOD, CAT and GSH-Px by using commercial diagnostic kits.

Statistical analysis

All values are presented as means \pm SE. Comparisons of data were made by one-way ANOVA. When data were significant, each group was compared with the others by Fisher's protected least significant difference test (StatView; SAS Institute, Cary, NC). Results with values of $P < 0.05$ were considered statistically significant.

RESULTS

At the end of 30 days, body weights in the groups 1 to 4 were 413.5 ± 8.4 , 407.8 ± 10.5 , 401.6 ± 8.7 and 414.8 ± 9.7 g, respectively, and no significant differences were detected among the four groups. As shown in Figure 1, the mean endurance times of running on a treadmill to exhaustion in the groups 2 to 4 were significantly prolonged compared with that in the group 1 ($P < 0.05$). As shown in Figure 2, MDA concentration of skeletal muscle of the groups 2 to 4 were significantly decreased compared with that of the groups 1 ($P < 0.05$). Table 1 shows the antioxidant enzyme activities. Compared with group 1, the antioxidant enzyme activities of SOD, GSH-Px and CAT of skeletal muscle of the groups 2 to 4 were all significantly higher.

DISCUSSION

This study aimed to examine the effects of AMP on oxidative damage in skeletal muscle of exhaustive exercise rats. The laboratory rat is a commonly used model for the evaluation of exhaustive exercise effects on biochemical changes in man. In this research, the mean

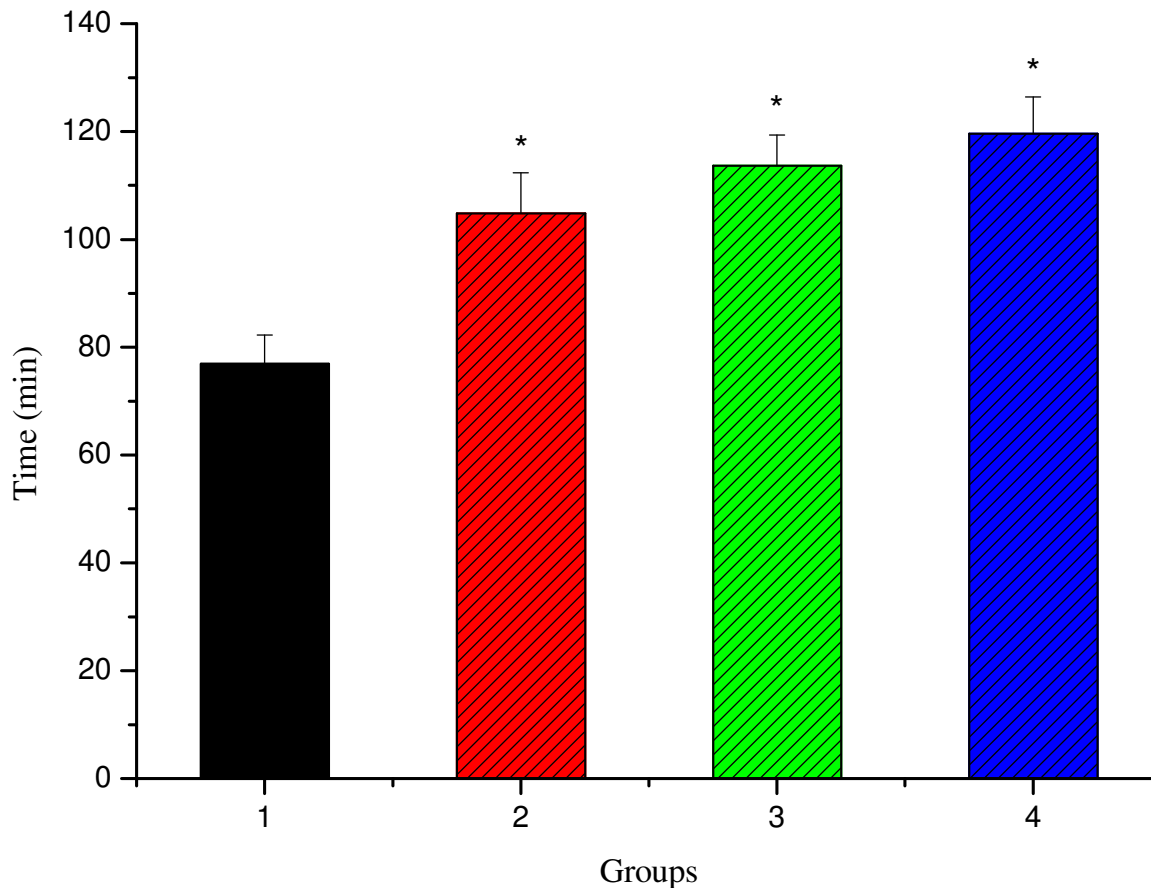


Figure 1. Effects of AMP on the endurance times of treadmill running to exhaustion $P < 0.05$, compared with the group 1. Eight rats were observed and tested for each group during the experimental period.

exhaustive exercise time of running on a treadmill in the groups 2 to 4 were significantly prolonged compared with that of the group 1 (Figure 1). It indicated that AMP supplementation could increase exhaustive time.

During exercise the need for oxygen increases. The process of delivering the oxygen to the working muscles may actually result in damage to polyunsaturated fatty acids in membrane structures (Hsieh et al., 2009), which has been documented by numerous investigations demonstrating increases in the byproducts of lipid peroxidation following exercise (Alessio and Goldfarb, 1988). When a hydroxyl radical reacts with an unsaturated fatty acid, a lipid peroxyl radical is formed. In the presence of oxygen this new free radical incites a chain of events referred to as lipid peroxidation. Lipid peroxidation of cell membranes results in decreased membrane fluidity, inability to maintain ionic gradients, cellular swelling, and tissue inflammation (Margaritis et al., 2003). MDA, a major end-product of oxidation of polyunsaturated fatty acids, has been frequently measured as an indicator of lipid peroxidation and oxidative stress *in-vivo*. Many studies have found acute exercise induced increases in MDA concentration in

tissues. In the present research, MDA concentration of skeletal muscle tissues of the groups 2 to 4 were significantly decreased compared with that of the group 1 (Figure 2). It indicated that AMP supplementation could effectively reduce lipid peroxidation to prevent oxidative damage in rat skeletal muscle.

Cells are equipped with a host of enzymes directly or indirectly involved in the antioxidant defense against ROS, providing primary defenses such as SOD, GSH-Px, and CAT. SOD, the most important enzyme present virtually in all aerobic organisms, catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. GSH-Px is a selenoenzyme which catalyzes the reduction of hydroperoxides at the expense of reduced glutathione. CAT is a primary antioxidant defense component that works to catalyze the decomposition of hydrogen peroxide to water, sharing this function with GSH-Px (Cotgreave et al., 1988). In this research, the antioxidant enzyme activities of SOD, GSH-Px and CAT of skeletal muscle of the groups 2 to 4 were significantly increased compared with that of the group 1 (Table 1). It indicated that AMP supplementation had beneficial effects on attenuating the oxidative stress

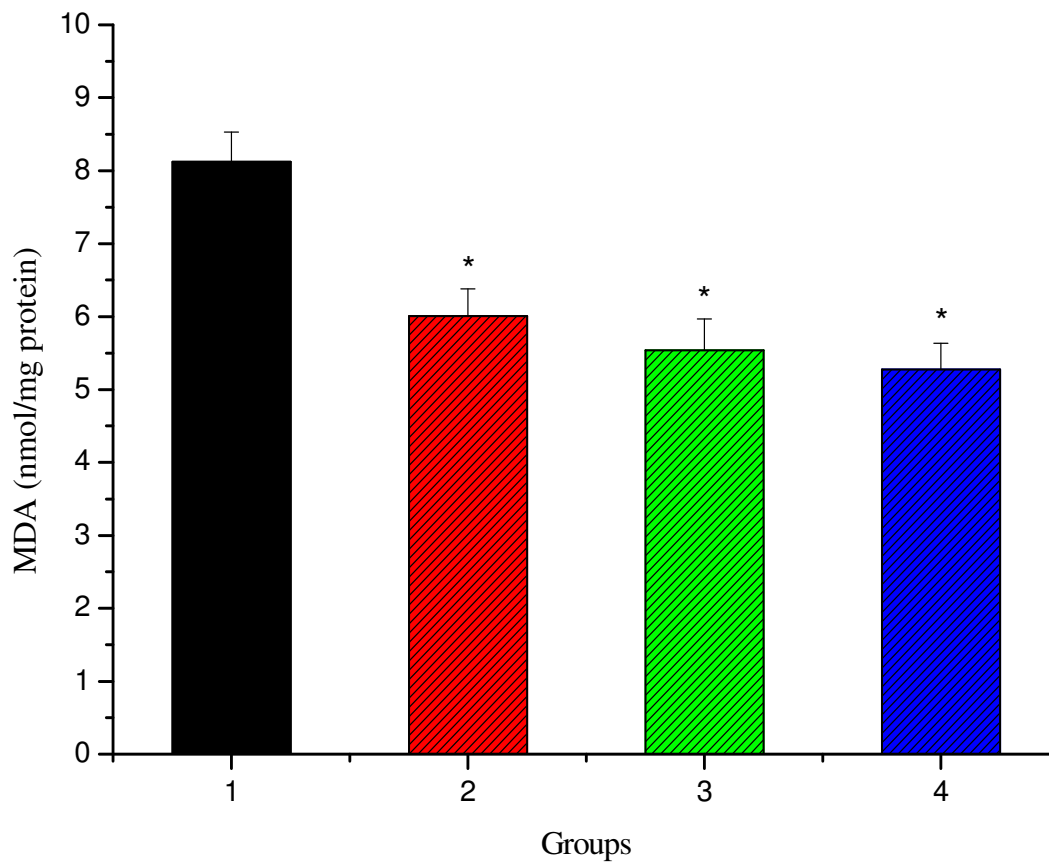


Figure 2. Effects of AMP on MDA concentration *P < 0.05, compared with the groups 1. Eight rats were observed and tested for each group during the experimental period.

Table 1. Effect of AMP on the activities of anti-oxidant enzymes in skeletal muscle of rats.

Groups	SOD (U/mg protein)	GPx (U/mg protein)	CAT (U/mg protein)
1	106.81±11.43	7.87±0.49	3.41±0.27
2	149.55±13.74*	9.01±0.93*	4.73±0.31*
3	161.62±9.91*	13.16±0.84*	5.76±0.37*
4	175.73±10.86*	15.87 ±1.19*	5.14±0.29*

*P<0.05, compared with the groups 1. Eight rats were observed and tested for each group during the experimental period.

induced by exhaustive exercise.

In conclusion, AMP supplementation increased performance of exhaustive exercise. And it is beneficial for increasing antioxidant status and inhibits oxidative stress induced by exhaustive exercise. It still needs further investigation and pathological observation in the future study.

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