



Protective effect of *Lycium barbarum* polysaccharides on oxidative damage in skeletal muscle of exhaustive exercise rats

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ABSTRACT

The aim of this study was to determine the modulatory effect of *Lycium barbarum* polysaccharides (LBP) on the oxidative stress induced by an exhaustive exercise. 32 male Wistar rats were taken in the study. The experiment was a 30-day exhaustive exercise program. We determined the lipid peroxidation, glycogen levels, and anti-oxidant enzyme activities in skeletal muscle. The results demonstrated that *L. barbarum* polysaccharides administration significantly increases glycogen level and anti-oxidant enzyme activities, and decreased malondialdehyde (MDA) level and creatine kinase activities. In conclusion, *L. barbarum* polysaccharides administration can significantly decrease the oxidative stress induced by the exhaustive exercise.

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1. Introduction

The beneficial effects of regular, nonexhaustive physical exercise have been known for a long time. There is irrefutable evidence of the effectiveness of regular physical activity in the primary and secondary prevention of several chronic diseases (e.g., cardiovascular disease, diabetes, cancer, hypertension, obesity, depression, and osteoporosis) and premature death [1]. However, the beneficial effects of exercise are lost with exhaustion. Exhausting [2–4] or moderate [5,6] exercise, in rats may increase reactive oxygen species (ROS) production exceeding the capacity of anti-oxidant defences. Oxidative stress is the imbalance of pro- and anti-oxidants in favor of the former. Exercise-induced oxidative stress was also reported in thoroughbred racehorses after a 1000-m race at maximum velocity [7]. Increased oxidative stress can be harmful to all cellular macromolecules, such as lipids, proteins, and DNA [8]. Some of this damage may be prevented by optimizing nutrition, particularly by increasing the dietary content of nutritional anti-oxidants [9]. In 1980, Koren et al. showed that free radical content was elevated in limb muscles stimulated to contract repetitively [10]. In 1999, Davies et al. found that there was free radical production in rat skeletal muscle after running until exhaustion [11]. Since then, research in the area has grown spectacularly.

Lycium barbarum belongs to the plant family Solanaceae. Red-colored fruits of *L. barbarum* have been used as a traditional Chinese herbal medicine for thousands of years [12]. The earliest known Chinese medicinal monograph, documented medicinal use of *L. barbarum* around 2300 years ago. *L. barbarum* polysaccharides (LBP) isolated from the red-colored fruits are the most important functional factor [13–18]. Five *L. barbarum* polysaccharides (LbGp1–LbGp5) were separated and structurally elucidated [19].

Polysaccharides act to protect polyunsaturated fatty acids in biological membranes against lipid peroxidation. There have been some reports on the anti-aging effects and fertility-promoting or anti-inflammatory effects of medicinal plant extracts [19,20], but few researchers have reported on the effects of plant polysaccharides on movement function. In the present study, we investigated the effects of polysaccharides supplementation on protective effects of LBP against oxidative damage in skeletal muscle of exhaustive exercise rats.

2. Materials and methods

2.1. Animals and exercise

A total of 32 male Wistar rats were obtained from experimental animal breeding center of our institute at 6 weeks of age. Rats were caged in groups of four or five under barrier conditions in the animal unit at our institute. They were all reared at 25 °C under artificial lighting for 12 h from 07:00 to 19:00 h and were given the standard laboratory diet and tap water ad libitum. The animals were

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cared for in accordance with the Guiding Principles for the Care and Use of Animals approved by the Council of the Physiological Society of China. All animals, at the beginning of the experiments, were divided randomly into the sedentary (group I) and exhaustive exercise (groups II–IV) groups. Each group contains eight animals.

All were administered orally and daily for 30 days. Group I received isotonic saline solution (ISS, 50 ml/kg body weight) as control (the sedentary group); Groups II–IV orally obtained 100, 200 and 300 mg/kg body weight of LBP in appropriate volumes of physiological saline, respectively, and allowed free access to standard laboratory pellet diet and water for 30 consecutive days.

The exhaustive experimental rats underwent a 30-day exhaustive exercise program. The run to exhaustion consisted of a single treadmill challenge at 35 m min⁻¹ and a slope of 9%. Rats were run at this speed and slope until they were unable to respond to continue prodding with a soft brush. The mean time to exhaustion was 100 min (range: 70–120 min).

2.2. Tissue preparations

About 48 h after the last session, rats were weighed and killed by decapitation. Hind-limb skeletal muscle was quickly excised and homogenized immediately with DY89-II homogenizer (NingBo Scientz Biotechnology Co. Ltd.) fitted with teflon plunger, in ice-chilled 10% KCl solution (10 ml/g of tissue). The suspension was centrifuged at 671 × g at 4 °C for 10 min and clear supernatant was used for the following estimations of activity of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), creatine kinase, and levels of malondialdehyde (MDA), glycogen by spectrophotometric methods.

2.3. Analytical method

Glycogen content in muscle tissue was determined in samples of frozen tissue (30 mg) by measuring amyloglucosidase-released glucose from glycogen by the method of Bergmeyer et al. [21] as previously described by Suzuki et al. [22].

The activity of creatine kinase was measured with an NADH-linked assay at 25 °C [23] and determined for the forward reaction (phosphagen synthesis) as described previously [24].

Lipid peroxidation was assayed by the measurement of the levels on the base of MDA, which was determined following the instructions on the kit. The content of MDA was expressed as nmol per gram protein. The assay for total SOD was based on its ability to inhibit the oxidation of oxyamine by the xanthine-xanthine oxidase system. Results were expressed as unit per gram muscle tissue.

The GPx activity assay was based on the method of Paglia and Valentine [25]. *tert*-Butylhydroperoxide was used as substrate. The assay measures the enzymatic reduction of H₂O₂ by GPx through consumption of reduced glutathione (GSH) that is restored from oxidized glutathione (GSSG) in a coupled enzymatic reaction by glutathione reductase (GR). GR reduces GSSG to GSH using NADPH as a reducing agent. The decrease in absorbance at 340 nm due to NADPH consumption was measured in a Molecu-

Table 1
Effect of polysaccharides administration on body weight of rats

Group	Initial body weight	Final body weight
I	189.67 ± 12.67	287.54 ± 30.83
II	187.54 ± 20.71	270.78 ± 24.05
III	195.37 ± 22.42	277.34 ± 18.75
IV	175.09 ± 14.62	291.39 ± 35.18

lar Devices M2 plate reader (Molecular Devices, Menlo Park, CA). GPx activity was computed using the molar extinction coefficient of 6.22 mM⁻¹ cm⁻¹. One unit of GPx was defined as the amount of enzyme that catalyzed the oxidation of 1.0 μmol of NADPH to NADP⁺ per minute at 25 °C.

2.4. Statistical analyses

All data in table are expressed as mean ± S.D. (*n* = 8) and differences between groups were assessed by analysis of variance (ANOVA) and Student's *t*-test. Differences were considered to be statistically significant if *P* < 0.05. All statistical analyses were carried out using SPSS for Windows, Version 11.5 (SPSS, Chicago, IL).

3. Results

3.1. Effect of polysaccharides administration on body weight of rats

After 30 days of exhaustive exercise, no significant change in body weight was noted in either group (Table 1).

3.2. Effect of polysaccharides administration on the level of MDA, muscle glycogen and activities of anti-oxidant enzymes in skeletal muscle of rats

This model of experimental exhaustive exercise promotes oxidative stress in skeletal muscle tissues of rats. This is confirmed by the decrease in muscle glycogen content and SOD, GPx activity (*P* < 0.01), and the increase in MDA concentration and creatine kinase activity in skeletal muscle tissues of exhaustive exercise animals (*P* < 0.01) with respect to controls (group I) (Table 2). Polysaccharides treatment is able to dose-dependently recover muscle glycogen levels and SOD, GPx activity in skeletal muscle tissues of exhaustive exercise animals, and statistically significant difference exists between exhaustive exercise control animals and polysaccharides-treated animals (*P* < 0.05 and *P* < 0.01). In addition, polysaccharides treatment significantly decreased MDA level and creatine kinase activity in a dose-dependent manner in skeletal muscle tissues of exhaustive exercise animals (*P* < 0.05 and *P* < 0.01) (Table 2).

Table 2
Effect of polysaccharides administration on the levels of MDA, muscle glycogen and activities of anti-oxidant enzymes in skeletal muscle of rats

Group	Muscle glycogen (mg/g protein)	SOD (U/mg protein)	GPx (U/mg protein)	MDA (nmol/mg protein)	Creatine kinase (U/L)
I	1.38 ± 0.11	205.4 ± 11.6	17.56 ± 1.67	3.89 ± 0.28	2107.43 ± 342.75
II	1.01 ± 0.12 ^a	104.9 ± 9.6 ^a	6.94 ± 0.51 ^a	7.59 ± 0.47 ^a	2783.57 ± 435.21 ^a
III	1.16 ± 0.09 ^b	146.4 ± 10.7 ^c	9.83 ± 0.79 ^c	6.03 ± 0.44 ^b	2414.23 ± 187.43 ^b
IV	1.22 ± 0.10 ^c	165.4 ± 13.2 ^c	15.39 ± 1.87 ^c	5.02 ± 0.63 ^c	2241.18 ± 305.16 ^c

^a *P* < 0.01, compared with normal control (group I).

^b *P* < 0.05, compared with exhaustive exercise control.

^c *P* < 0.01, compared with exhaustive exercise control.

4. Discussion

Exercise promotes physiological changes in response to disturbances in cellular homeostasis. These changes do not cease until a small overcompensation is attained, demonstrating that exercise is an excellent model to study physiological stress and the adaptive capacity of the body. An optimal balance between training and recovery is also necessary for improvement in athletic performance [26].

The idea of the deleterious effects of free radicals has been firmly entrenched in the minds of scientists for the past 30 years. However, there is now an appreciation that the ROS generated during muscle contraction have a physiological role in the adaptation to exercise. In response to the free radical assault, the cell has developed a number of anti-oxidant defense systems such as superoxide dismutase, the peroxidases, the glutathione redox cycle with its associated constitutive enzymes, as well as glutathione itself, whose concentration is higher in the cell than that of glucose [27]. Therefore the cell has become well equipped to deal with the normal production of ROS. Exhaustive exercise-induced muscle damage has been widely reported [28,29].

Our study showed, as indicators for oxidative stress during the exhaustive exercises, higher levels of MDA and activity of creatine kinase, and decreased levels of glycogen and activity of SOD and GPx. Lipid peroxidation causes a loss in fluidity and an increase in the permeability of membranes, resulting in loss of cytosolic proteins. It has been suggested that a low anti-oxidant enzyme activities together with high levels of MDA, as in this work, could indicate that there is a relationship between the free radical attack and the exhaustive exercise [30]. The sport population is usually supplemented with high levels of anti-oxidants. In the present study, we found a higher increase in the SOD, GPx activities and glycogen levels just after polysaccharides administration. Furthermore, in this work, we have found a significant decrease in the MDA content and creatine kinase activity after polysaccharides administration. This result is in agreement with Li et al.'s work [31].

In summary, a long strenuous bout of exercise can cause a significant increase in oxidative stress. Furthermore, 1 month of polysaccharides supplementation significantly decreased oxidative stress. Our results indicate that polysaccharides supplementation is effective in avoiding oxidative stress after intense long-duration exercise.

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