Lycium barbarum Polysaccharides Protects against Strenuous Exercise-induced Oxidative Damage in Rats

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Keywords: *Lycium Barbarum* Polysaccharides, Oxidative Damage, Exhaustive Running Exercise, Rats.

Abstract: *Lycium barbarum* contains a variety of nutrients and bioactive ingredients, which has multiple biological and pharmacological effects. In the theory of Chinese medicine, *Lycium barbarum* as a common Chinese herbal medicine can be used for the treatment of many diseases. The aim of the current study was to evaluate the protective effects of *Lycium barbarum* polysaccharides (LBPs) on strenuous exercise-induced oxidative damage in rats. The animals were divided into one control group (treated with distilled water) and three LBPs groups (treated with 50, 100 and 200 mg/kg LBPs, respectively). After 28 days of treatment, the exhaustive running exercise was performed, followed by the relevant biochemical parameter analysis. the datas revealed that LBPs increases exhaustive running times, and levels of superoxide dismutase (SOD), glutathione peroxidase (GPx), reduced glutathione (GSH), glutathione reductase (GR), and catalase (CAT) in liver. LBPs decreases the levels of creatine kinase (CK), myoglobin (Mb), tumor necrosis factor-α (TNF-α) and interleukin 1β (IL-1β) in serum, and also the levels of oxidized glutathione (GSSG), malondialdehyde (MDA) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) in liver. These results suggest that LBPs has protective effects on oxidative damage induced by strenuous exercise.

1 INTRODUCTION

Reactive oxygen species (ROS) are the chemically active oxygen free radicals and the substances that can be converted to the free radicals, which are generated during the aerobic metabolism of cells. ROS mainly includes oxygen free radicals and some non-free radicals substances such as hydrogen peroxide (H_2O_2), hydroperoxide (ROOH), etc. (Kurutas 2016). Under normal physiological conditions, the body's antioxidant defense systemcan remove ROS, maintaining a dynamic balance. However, strenuous exercise can increase oxygen intake, accompanied by the generation of ROS in various tissues by mean of different ways, which may exceed the capacity of defense system, resulting in increased oxidative stress. Exercise-induced endogenous ROS were produced by a variety of sources, including mitochondrial respiratory chain pathway, xanthine oxidase reaction pathway, neutrophils respiratory burst pathway, hemoglobin oxidation reaction pathway, and so on. It has been reported that increased oxidative stress can bring about various levels of oxidative damage to various substances that make up cell tissue such as lipids, sugars, proteins and DNA (Jówko et al. 2011). Growing evidences show that exogenous antioxidants from food and natural products supplementation can be an effective means to cut down exercise-induced oxidative damage (Chen et al. 2013).

Lycium barbarum (*L. barbarum*) is a perennial woody plant, mainly grown in some provinces of northern China, such as Inner Mongolia, Ningxia and Hebei. The fruits of *L. barbarum*, known as wolfberry and Goqi, have been widely used as traditional herbs and supplements more than 2500 years. In 2002, the fruits of *L. barbarum* were identified as both food and medicine items by the Chinese government departments. Many types of components, such as polyphenols, polysaccharides, alkaloids, carotenoids, vitamins, amino acids, aminoethanesulfonic acids, and fatty acids in fruits of L. barbarum have been isolated and identified, which have some biological and health-related activity (Tang et al. 2015). Numerous studies have suggested that *L. barbarum* polysaccharides (LBPs) is the most important ingredients of L. barbarum to play many biological activities (Liu et al. 2015). LBPs account for about 5 - 8% of the dry weight of

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fruits of *L. barbarum*, which is a heteropolysaccharide containing protein, and generally consists of 6 - 8 monosaccharides, 18 amino acids and a variety of trace elements. The molecular weight is in the range of 24 to 241 kDa (Chen et al. 2009). Modern pharmacological studies indicated LBPs has multiple pharmacological and biological functions, including anti-diabetic, anti-hypoxia, anti-fatigue, hypolipidemic, antihypertensive, anti-aging, anti-cancer, analgesic, immune regulation and liver protection effects. Especially, this compound exhibited strong antioxidant activities in vitro by inhibiting different types of free radicals (DPPH, ABTS, superoxide anion and hydroxyl radical), reducing power activities and metal ion chelating capability (Li and Zhou 2007). Animal experiments also indicated that LBPs can significantly lower lipid peroxidation and improve antioxidant enzyme activities (Zhao et al. 2015). Based on the antioxidant activities of LBPs, it can be hypothesized that strenuous exercise-induced oxidative damage in animal model can be prevented by LBPs pretreatment. Thence, the research was implemented to investigate whether LBPs administration could prevent strenuous exercise-induced oxidative damage in rats.

2 EXPERIMENTAL

2.1 Plant Material

The dried fruits of *L. barbarum* were collected in Zhong-ning County of Ning Xia Huizu Autonomous Region and provided by Qinian Biological Technology Co., Ltd. (Yinchuan, China). The plant samples were authenticated by a biologist in the college of chemistry and chemical engineering, Central South University (Changsha, China). The voucher specimen was laid in plants herbarium of Central South University.

2.2 Chemicals and Reagents

Commercial diagnostic kit for creatine kinase (CK) was provided by Suzhou Comin Technology Co. (Suzhou, China). Commercial diagnostic kits for reduced glutathione (GSH), oxidized glutathione (GSSG) and glutathione reductase (GR) were provided by Beyotime Biotechnology Institute (Haimen, China). Commercial diagnostic kits for catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MDA), and glutathione peroxidase (GPX) were provided by Jiancheng Research institutions (Nanjing, China). ELISA kits for myoglobin (Mb) were provided by Huamei Biological Engineering Co. (Wuhan, China). ELISA kits for tumor necrosis factor-α (TNF-α), interleukin 1β (IL-1β) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) were provided by Huijia Biological Technology Co. (Xiamen, China).

2.3 Experimental Animals

Male Wistar rats (weight 180 - 200 g) adapt to the environment and diet for one week before the experiment. During the experiment, four rats were placed in an individual plastic cage under standard feeding conditions (temperature of 22 ± 2 °C, relative humidity of 50 ± 15%, and 12 h light: 12 hours dark cycle circulation). Animals ingested commercial rodent food and free drinking purified water. This animal experiment was approved by the Ethics Committee of Central South University.

2.4 Preparation of *L. barbarum* Polysaccharides

L. barbarum polysaccharides (LBPs) were extracted according to the previously published method in the literature (Zhao et al. 2005), and have been slightly adjusted. Briefly, the dried samples were crushed to fine powder with electric mill and passed the 200 mesh sieve. Then the powder was refluxed twice with petroleum ether (1 h every time) to remove the lipid, and then refluxed twice with 80% ethanol (1 h every time) to remove the small molecule sugar. The residue was extracted with 10 volumes of distilled water at 90 °C for three times (2.5 h every time). The filtrate from combined and filtered water extracts was concentrated in a rotary evaporator under reduced pressure at 50 °C. Then the concentrate was centrifuged (3000 rpm, 15 min), and the supernatant was mixed with 4 volumes of 95% ethanol and stockpiled overnight at 4 °C. The precipitation was washed in order with anhydrous ethanol, acetone and ether, and the reagent was evaporated. The resulting precipitate was dispersed in distilled water, dialyzed and lyophilized to afford crude polysaccharides.

2.5 Experimental Design

Animals had one week adaption period, and after that, they were divided into four groups, each consisting of 8 rats. LBPs were given to the rats at doses of 0, 50, 100 and 200 mg/kg and the four groups were accordingly named as the control (C)

group, the low-dose LBPs treatment (LBPL) group, the medium-dose LBPs treatment (LBPM) group and the high-dose LBPs treatment (LBPH) group. LBPs were dissolved in 1.0 mL distilled water and administered by oral gavage one time per day lasting for 28 days. After 21 days, the rats were introduced to the motor-driven treadmill (WI78059, Shanghai Yuyan Scientific Instrument Co., Ltd.) and made to run at 15 m/min and a 0° grade for 15 min one time per day lasting for 7 days to accommodate running exercise. At the final day of experiment (the 28th day), the incremental running exercise to exhaustion was conducted using methods previously described (Huang et al. 2013) with some modifications. The rats were introduced into a treadmill, started running at 15 m/min and 0 °grade for 10 min, then at 20 m/min and 0 °grade for 10 min, and finally at 30 m/min and 10 ° grade to exhaustion. Exhaustion is defined when the rats can't continue running on the treadmills after 12 s of continuous electric shock, and the exhaustive running time was measured.

2.6 Analysis of Biochemical Parameters

After exhaustive running exercise, the rats were sacrificed by decapitation under ether anesthesia. Blood was collected and centrifuged (3000 rpm, 15 min) at 4 °C to obtain serum for CK, Mb, TNF-α and IL-1β analysis. The liver samples were immediately isolated, weighed, and homogenized for GSH, GSSG, SOD, CAT, GPX, GR, MDA and 8-OHdG determinations. The levels of CK, Mb, TNF-α, IL-1β, SOD, CAT, GPX, GR, GSH, GSSG, MDA and 8-OHdG were determined using commercial assay kits and abiding by the procedures advised by manufacturers.

2.7 Statistical Analysis

All data were presented as mean ± standard deviation (SD), and SPSS software is used for Statistical analysis.

3 RESULTS AND DISCUSSION

3.1 Effects of LBPs on the Exhaustive Running Times

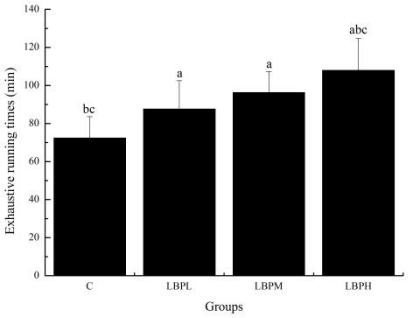

Figure 1: Effects of LBPs on the exhaustive running times.

According to Figure 1, the exhaustive running time of different doses of LBP groups (LBPL, LBPM and LBPH) were significantly longer than that of C group ($p < 0.05$). Compared with LBPL group, exhaustive running time of LBPH group was significantly prolonged ($p < 0.05$). Compared with LBPM group, the exhaustive running time of LBPH group was significantly prolonged ($p < 0.05$). The above data showed that LBPs had a strong anti-fatigue effect.

3.2 Effects of LBPs on the CK and Mb in Serum

Strenuous exercise leads to increased muscle membrane permeability or muscle cell damage, causing creatine kinase (CK) and myoglobin (Mb) and other proteins to escape from the cell and into the blood circulation.

According to Figure 2, the CK and Mb levels of the different doses of LBP groups (LBPL, LBPM and LBPH) were significantly reduced compared with the C group ($p < 0.05$). The CK levels of the LBPH groups, as well as the Mb levels of the LBPM and LBPH groups were significantly reduced compared with the LBPL group ($p < 0.05$). The CK and Mb levels of the LBPH groups were significantly reduced compared with the LBPM group ($p < 0.05$). The above data showed that LBPs might prevent muscle damage or promote rapid regeneration of damaged muscle.

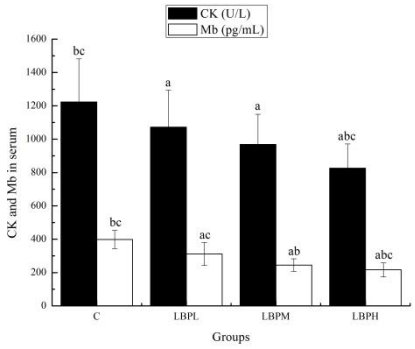

Figure 2: Effects of LBPs on the CK and Mb in serum.

3.3 Effects of LBPs on the TNF-α and IL-1β in Serum

Previous studies have shown that strenuous exercise can induce proinflammatory cytokines and pleiotropic cytokine secretion to increase. TNF-α and IL-1β are inflammatory cytokines secreted by monocyte-macrophages and have proinflammatory effects, which could also stimulate the production of pleiotropic cytokine IL-6.

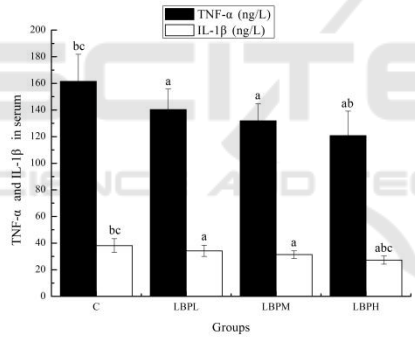

Figure 3: Effects of LBPs on TNF-α and IL-1β in serum.

According to Figure 3, the TNF-α and IL-1β levels of LBPL, LBPM and LBPH were significantly reduced compared with the C group ($p < 0.05$). Compared with the LBPL group, the TNF-α and IL-1β levels of the LBPH groups were significantly reduced ($p < 0.05$); Compared with the LBPM group, the IL-1β levels of the LBPH groups were significantly reduced ($p < 0.05$). The above data showed that LBPs could attenuate strenuous exercise-induced inflammatory responses.

3.4 Effects of LBPs on the SOD, CAT, GPX and GR in Liver

Antioxidant enzymes play an important protective role in exercise-induced free radical oxidative damage, and the decrease in the activity of these enzymes means that the tissue is more susceptible to free radical damage (Lee et al. 2009).

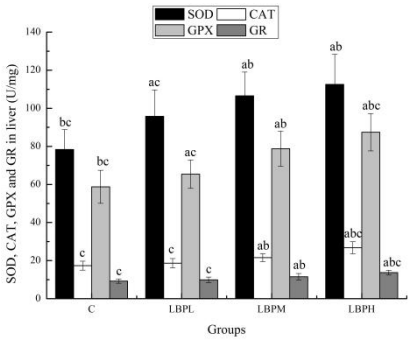

Figure 4: Effects of LBPs on the SOD, CAT, GPX and GR in liver.

According to Figure 4, the SOD and GPX levels of the LBPL, LBPM and LBPH groups; the CAT and GR levels of the LBPM and LBPH groups were significantly prolonged compared with the C group ($p < 0.05$). Compared with the LBPL group, the SOD, CAT, GPX and GR levels of the LBPM and LBPH groups were significantly prolonged ($p < 0.05$). Compared with the LBPM group, the CAT, GPX and GR levels of the LBPH groups were significantly prolonged ($p < 0.05$). The above data showed that LBPs could up-regulate the expression of antioxidant enzymes to prevent strenuous exercise-induced oxidative damage.

3.5 Effects of LBPs on the GSH and GSSG in Liver

Glutathione is a tripeptide consisting of glutamine, cysteine and glycine, which has two forms of reduced (GSH) or oxidized (GSSG) (Masella et al. 2005). As a main intracellular antioxidant, GSH plays an important role in preventing exercise-induced oxidative damage by removing free radicals and preventing the accumulation of hydroperoxides. Under the action of GPx, GSH can reduce the H_2O_2 to produce H_2O, while GSH is oxidized to GSSG in cells. GSSG also produces GSH under the catalysis of GR. Strenuous exercise can cause severe oxidative stress, leading to accumulation of GSSG and reduction of GSH. GSH depletion can increase the formation of hydroxyl radicals, which would further lead to DNA damage (Muñoz et al., 2010). Therefore, depletion of GSH in tissues has been used as a sensitive index of exercise-induced ROS production.

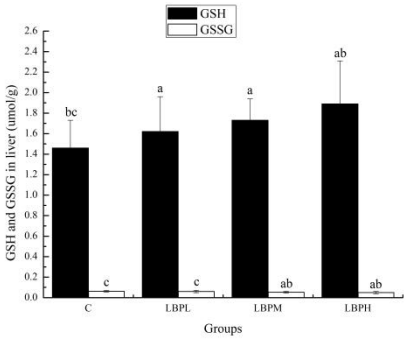

Figure 5: Effects of LBPs on the GSH and GSSG in liver.

According to Figure 5, compared with the C group, the GSH levels of the LBPL, LBPM and LBPH groups were significantly prolonged ($p < 0.05$); the GSSG levels of the LBPM and LBPH groups were significantly reduced ($p < 0.05$). Compared with the LBPL group, the GSH levels of the LBPH groups were significantly prolonged ($p < 0.05$); the GSSG levels of the LBPM and LBPH groups were significantly reduced ($p < 0.05$). The above data showed that LBPs are adequate protection against exercise-induced ROS generation.

3.6 Effects of LBPs on the MDA and 8-OHdG in Liver

MDA is the end product of peroxidative decomposition of polyenic fatty acids and has been often used as a marker of lipid peroxidation. 8-OHdG is one of the major products of DNA oxidation. When ROS attacks the guanine in DNA, it causes deoxyguanosine oxidation to form 8-OHdG. 8-OHdG can be measured with high sensitivity and it is extensively investigated in human and animal exercise studies, and is thus used as a biomarker of oxidative DNA damage (Hamurcu et al. 2010).

According to Figure 6, the MDA and 8-OHdG levels of the LBPL, LBPM and LBPH groups were reduced compared with the C group ($p < 0.05$). Compared with the LBPL group, the MDA and 8-OHdG levels of the LBPM and LBPH groups were significantly reduced ($p < 0.05$). Compared with the LBPM group, the MDA levels of the LBPH groups were significantly reduced ($p < 0.05$). The above data showed that LBPs could attenuate lipid peroxidation and oxidative DNA damage induced by exhaustive exercise.

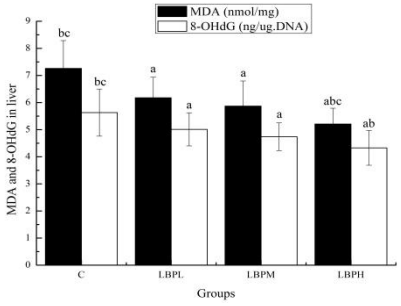

Figure 6: Effects of LBPs on the MDA and 8-OHdG in liver.

4 CONCLUSIONS

The results of this study provide strong evidence that LBPs have protective effects on oxidative damage induced by strenuous exercise in rats due to increased the levels of SOD, CAT, GPX, GR and GSH in liver, simultaneously decreased the levels of CK, Mb TNF-α and IL-1β in serum, and the levels of GSSG, MDA and 8-OHdG in liver. The protective effects on oxidative damage of LBPs was dose-dependent in rats, which might be related to the per se antioxidant activities of LBPs.

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