

Full Length Research Paper

Supplementation of *Lycium barbarum* polysaccharides protection of skeletal muscle from exercise-induced oxidant stress in mice

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The present study aimed to determine whether *Lycium barbarum* polysaccharides (LBP) could limit oxidative stress induced by swimming exercise. The mice were randomly assigned to four groups, that is, one normal control group and three LBP treatment groups. LBP treatment groups (I, II and III) were administered with three different doses of LBP: 100, 200 and 300 mg/kg/day by gavage for 30 days. The normal control group was given vehicle alone by oral gavage for 30 days. At the end of the 30 days treatment, forced-swimming test was performed and mean swim-to-exhaustion time of mice was immediately recorded. Then, the contents of malondialdehyde (MDA), super oxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) of gastrocnemius muscle were determined. The results showed that LBP prolonged exhaustive swim time and improved exercise tolerance. Meanwhile, LBP promote increases in the activities of main antioxidant enzymes (SOD, CAT and GPx) and attenuated MDA increase. This suggests that supplementation of *L. barbarum* polysaccharides protects skeletal muscle from exercise-induced oxidant stress in mice.

Key words: *Lycium barbarum* polysaccharides, exercise, oxidant stress, mice.

INTRODUCTION

Several studies have demonstrated that exhaustive exercise is associated with accelerated generation of reactive oxygen species (ROS) that results in oxidative stress (Zhang et al., 2004; Powers and Jackson, 2008; Motta et al., 2009; Smarsh et al., 2010), which may impair liver, kidney, skeletal muscle and other tissues (Morillas-Ruiz et al., 2005). The antioxidant system is used to protect organism from harmful effects of free radicals. This system consists of antioxidant enzymes (catalase, glutathione peroxidase and superoxide dismutase) and non-enzymatic antioxidants (vitamin E, vitamin A, vitamin C, glutathione and uric acid). The imbalance between free radical production and

antioxidant defense leads to an oxidative stress state (Çiçek, 2006; Lambertucci et al., 2007). Therefore, to avoid or minimize deleterious effects of exercise induced oxidative stress, the antioxidant capacity of the cell must be increased. Therefore, this increased capacity may be achieved through appropriate training, diet and most importantly through the use of antioxidant supplementation (Bing and Zhaobao, 2010).

Lycium barbarum (Chinese Name: Gou qi) belongs to the plant family Solanaceae (Li et al., 2007; Zhao et al., 2009). Red-colored fruits of *L. barbarum* have been used as a traditional Chinese herbal medicine for thousands of years, and which have a large variety of biological activities and pharmacological functions and play an important role in preventing and treating various chronic diseases, such as diabetes, hyperlipidemia, cancer, hepatitis, hypo-immunity function, thrombosis, fatigue and male infertility (Luo et al., 2004; Wang et al., 2006; Chao et al., 2006; Chan et al., 2007; Amagase et al., 2009;

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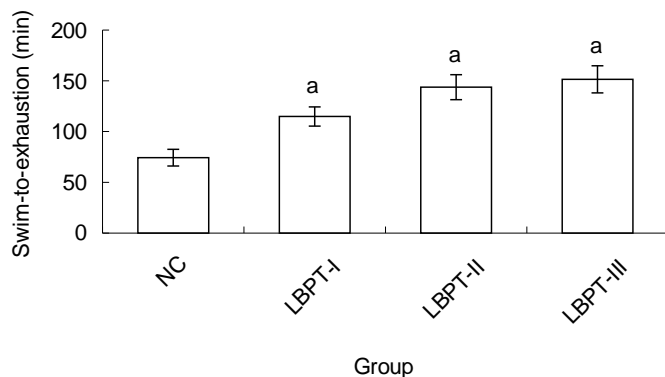


Figure 1. Effect of LBP on mean swim-to-exhaustion time of mice. ^aP < 0.05, compared with normal control group (NC).

Potterat, 2010). Previous studies have shown that *L. barbarum* polysaccharides (LBP) is one of the major active components. Its multiple pharmacological effects, such as antitumor, anti-aging, anti-diabetic, anti-fatigue, anti-cancer and anti-oxidant have been demonstrated in many animal models *in vivo* and *in vitro* (Wang and Ng, 1999; Gan et al., 2004; Ni et al., 2004; Li et al., 2007; Zhao et al., 2009; Lin et al., 2009; Yao and Li, 2010; Mao et al., 2011). However, the effects of LBP on the exercise-induced oxidative stress in mice have not been investigated. Hence, the present study aimed to determine whether LBP could limit oxidative stress induced by swimming exercise.

MATERIALS AND METHODS

Dried *L. barbarum* fruits was purchased from a local market (Jinan, China) and identified by a botanist. The kits for malondialdehyde (MDA), super oxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were purchased from Jiancheng Bioengineering Institute (Nanjing, China). All the other chemicals used were of analytical grade.

Ethics statement

All animals were handled in strict accordance with good animal practice as defined by the relevant local animal welfare bodies, and all animal work was approved by the Animal Research Committee of the Shandong University of Science and Technology (Jinan, China).

Preparation of *L. barbarum* polysaccharides

Dried *L. barbarum* fruits were ground to a fine powder. Polysaccharides were prepared by the method of Luo et al. (2004) and Ma et al. (2009) with slight modification. In brief, *L. barbarum* fruits powder (100 g) were placed in 1.5 L of boiling water and decocted for 3 h. The decoction was left to cool at room temperature, filtered and then freeze-dried to obtain crude polysaccharides. The dried crude polysaccharides were refluxed three times to remove lipids with chloroform/methanol (2:1, v/v). After filtering, the residues were air-dried and then refluxed again with 80% ethanol. The

resultant product was extracted three times in hot water (90°C) and then filtered. The combined filtrate was precipitated using 95% ethanol, 100% ethanol and acetone, respectively. After filtering and centrifuging, the precipitate was collected and vacuum-dried, affording the desired LBP.

Tested animals

Male Kunming mice weighing 20 ± 2 g were purchased from Libo Laboratory Animal Breeding Center (Jinan, China). The animals were housed in polypropylene cages and maintained under controlled conditions of 12 h light/12 h dark cycle and 50% relative humidity at 25 to 30°C. The animals were fed pellet diet and water *ad libitum*.

Experimental design

After a period of 1 week, the animals were randomly assigned to four groups: normal control group (NC), LBP treatment group I (LBPT-I), LBP treatment group II (LBPT-II) and LBP treatment group III (LBPT-III) (n = 10 in each group). LBP treatment groups (I, II and III) were administered three different doses of LBP: 100, 200 and 300 mg/kg/day by gavage for 30 days. The normal control group was given vehicle alone by oral gavage for 30 days.

At the end of the 30 days treatment, forced-swimming test was performed by a method described previously (Chi et al., 2008). In brief, the mice swam with wire of 5% body weight tied to their tails in the pool (length: 65 cm, width: 50 cm, depth: 50 cm) filled with 30 cm depth of water at 30 to 35°C. Mice were regarded as exhaustion when they were underwater for 8 s and the mean swim-to-exhaustion time was immediately recorded. After the forced-swimming test, the mice were anesthetized with an intraperitoneal injection of ketamine (80 mg/kg) and xylazine (4 mg/kg). After anesthetization, the mice were killed by decapitation and the gastrocnemius muscle were excised, weighed and immediately frozen at 70°C to determine MDA, SOD, CAT and GPx contents. The contents of MDA, SOD, CAT and GPx were determined using commercially available kits according to the manufacturers' instructions.

Statistical analysis

All values are expressed as mean ± standard deviation. Statistical comparisons were made by one-way analysis of variance (ANOVA), and correlation analysis was performed by Pearson product moment using Statistical Package for Social Sciences (SPSS) version 13.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was defined as P < 0.05.

RESULTS AND DISCUSSION

Effect of LBP on mean swim-to-exhaustion time of mice

A forced-swimming test was used to evaluate the extent of fatigue, which increased the exercise intensity of mice in order to shorten the investigational time. With 6 to 8% body weight load, the mice could swim freely and safely (Wang et al., 2008). In this experiment, the mice had a weight attached, 5% body weight in the duration of the swim-to-exhaustion. As shown in Figure 1, the mean

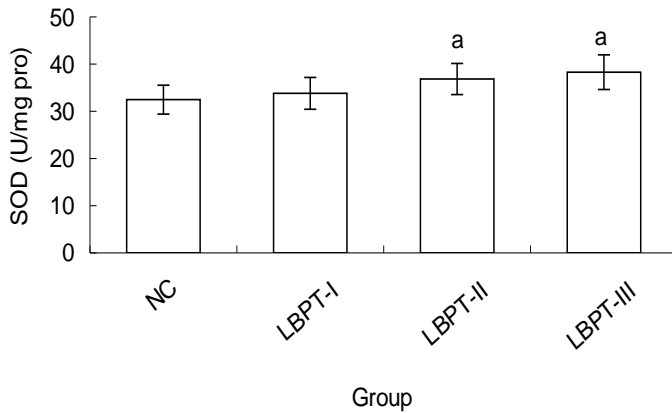


Figure 2. Effect of LBP on SOD contents of mice. ^aP < 0.05, compared with normal control group (NC).

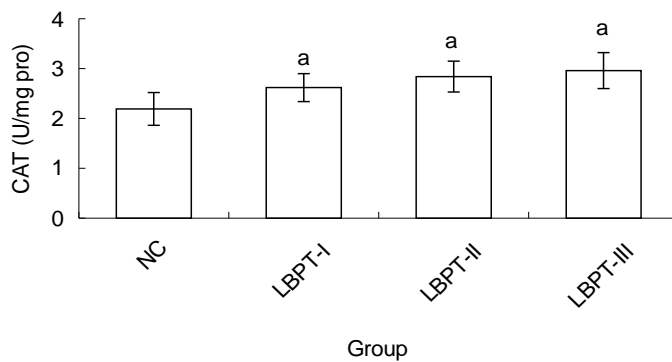


Figure 3. Effect of CAT on SOD contents of mice. ^aP < 0.05, compared with normal control group (NC).

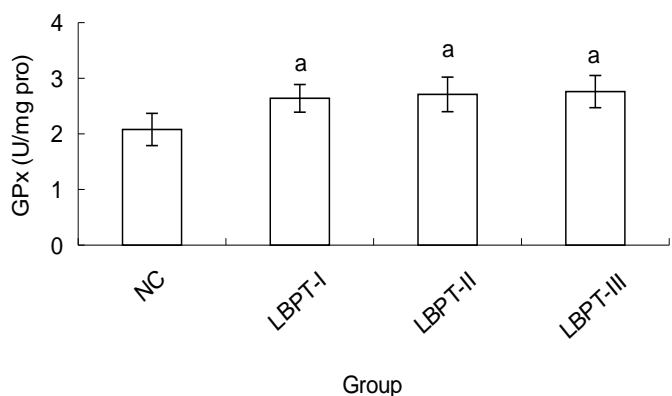


Figure 4. Effect of GPx on SOD contents of mice. ^aP < 0.05, compared with normal control group (NC).

swim-to-exhaustion time of LBP treatment groups (I, II and III) was longer ($P < 0.05$) than for the normal control group, while the difference between LBPT-II and LBPT-III

was not significant ($P > 0.05$). The present study demonstrated that LBP prolonged exhaustive swim time and improved exercise tolerance.

Effect of LBP on SOD, CAT and GPx contents of mice

It is well known that primary antioxidant enzymes include SOD, GPx and CAT. SOD dismutates superoxide radicals to form H_2O_2 and O_2 . GPx is an enzyme responsible for reducing H_2O_2 or organic hydroperoxides to water and alcohol, respectively. CAT catalyses the breakdown of H_2O_2 to form water and O_2 (Shan et al., 2011). Evidence showed that decrease in the contents of SOD, GPx and CAT in the muscle tissue after exhaustive exercise may be an indication of exercise-induced oxidant stress threat (Misra et al., 2009). As shown in Figure 2, the SOD contents of LBP treatment groups (II and III), increased ($P < 0.05$) than for the normal control group. As shown in Figure 3, the CAT contents of LBP treatment groups (I, II and III) increased ($P < 0.05$) than for the normal control group. As shown in Figure 4, the GPx contents of LBP treatment groups (I, II, III) increased ($P < 0.05$) than for the normal control group. The present study demonstrated that LBP promotes increase in the activities of these antioxidant enzymes (SOD, CAT and GPx), which had beneficial effects on attenuating the oxidative stress induced by exhaustive exercise.

Effect of LBP on MDA contents of mice

Exhaustive exercise is known to induce the generation of free radicals. This increased level of free radicals might cause an increase in lipid peroxidation (Skarpańska-Stejnborn et al., 2010). One approach to study oxidative stress is to measure the peroxidation of lipids. Lipid peroxidation is the end result of damaging radical chain reactions that usually begin with a single hydrogen abstraction from an unsaturated fatty acid (Sachdev and Davies, 2008). Following the abstraction of hydrogen, the original radical (R) is neutralized and a lipid radical (L) is generated by oxygen to generate a lipid peroxy radical (LOO).

This highly unstable radical can then react further with neighboring fatty acids, in a self-propagating chain reaction. This can then cause damage to the integrity of cell membranes, endoplasmic reticulum membranes and nucleus membranes (Reid et al., 1994). This lipid peroxidation leads to the formation of a wide array of primary oxidation products (e.g. conjugated dienes or lipid hydroperoxides) and secondary oxidation products, including lipid aldehydes, such as MDA and alkanes. MDA have been frequently used as markers of oxidative stress in response to exercise (Urso and Clarkson, 2003). There are many studies that describe increase in the contents of MDA in skeletal muscle, liver and plasma after exhaustive exercise (Vani et al., 1990; Leeuwenburgh and Ji, 1998;

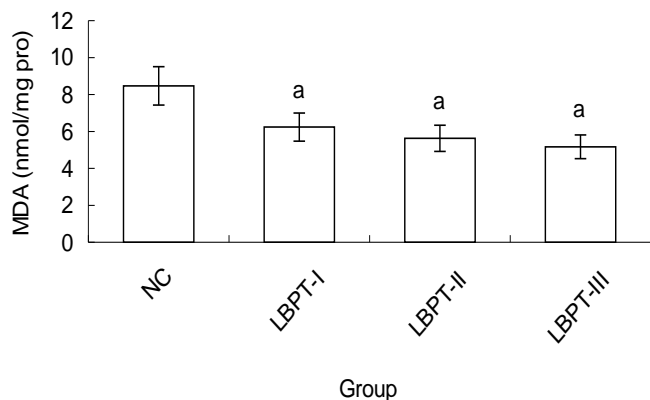


Figure 5. Effect of LBP on MDA contents of mice. ^aP < 0.05, compared with normal control group (NC).

Zhang et al., 2003; Kon et al., 2007; Huang et al., 2008). As shown in Figure 5, the MDA contents of LBP treatment groups (I, II and III) decreased ($P < 0.05$) than for the normal control group. The present study demonstrated that LBP attenuated MDA increase, which could effectively reduce lipid peroxidation.

Conclusion

Conclusively, the present study clearly indicates that LBP prolonged exhaustive swim time and improved exercise tolerance. Meanwhile, LBP promotes increase in the activities of main antioxidant enzymes (SOD, GPx and CAT) and attenuated MDA increase, which could effectively reduce lipid peroxidation. This suggests that supplementation of *L. barbarum* polysaccharides protects skeletal muscle from exercise-induced oxidant stress in mice.

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REFERENCES

- Amagase H, Sun B, Nance DM (2009). Immunomodulatory effects of a standardized *Lycium barbarum* fruit juice in Chinese older healthy human subjects. *J. Med. Food*, 12: 1159-1165.
- Bing Y, Zhaobao W (2010). Effects of Ginkgo biloba extract on free radical metabolism of liver in mice during endurance exercise. *Afr. J. Tradit. Complement. Altern. Med.*, 7: 291-295.
- Chan HC, Chang RC, Koon-Ching Ip A, Chiu K, Yuen WH, Zee SY, So KF (2007). Neuroprotective effects of *Lycium barbarum* Lynn on protecting retinal ganglion cells in an ocular hypertension model of glaucoma. *Exp. Neurol.*, 203: 269-273.
- Chao JC, Chiang SW, Wang CC, Tsai YH, Wu MS (2006). Hot water-extracted *Lycium barbarum* and *Rehmannia glutinosa* inhibit proliferation and induce apoptosis of hepatocellular carcinoma cells. *World J. Gastroenterol.*, 12: 4478-4484.
- Chi AP, Chen JP, Wang ZZ, Xiong ZY, Li QX (2008). Morphological and structural characterization of a polysaccharide from *Gynostemma pentaphyllum* Makino and its anti-exercise fatigue activity. *Carbohydr. Polym.*, 74: 868-874.
- Çiçek D (2006). Exercise and oxidative stress. *Anadolu Kardiyol Derg.*, 6: 141-142.
- Gan L, Hua Zhang S, Liang Yang X, Bi Xu H (2004). Immunomodulation and antitumor activity by a polysaccharide-protein complex from *Lycium barbarum*. *Int. Immunopharmacol.*, 4: 563-569.
- Huang CC, Tsai SC, Lin WT (2008). Potential ergogenic effects of L-arginine against oxidative and inflammatory stress induced by acute exercise in aging rats. *Exp. Gerontol.*, 43(6): 571-577.
- Kon M, Kimura F, Akimoto T, Tanabe K, Murase Y, Ikemune S, Kono I (2007). Effect of Coenzyme Q10 supplementation on exercise-induced muscular injury of rats. *Exerc. Immunol. Rev.*, 13: 76-88.
- Lambertucci RH, Levada-Pires AC, Rossoni LV, Curi R, Pithon-Curi TC (2007). Effects of aerobic exercise training on antioxidant enzyme activities and mRNA levels in soleus muscle from young and aged rats. *Mech. Ageing Dev.*, 128(3): 267-275.
- Leeuwenburgh C, Ji LL (1998). Glutathione and glutathione ethyl ester supplementation of mice alter glutathione homeostasis during exercise. *Free J. Nutr.*, 128(12): 2420-2426.
- Li XM, Ma YL, Liu XJ (2007). Effect of the *Lycium barbarum* polysaccharides on age-related oxidative stress in aged mice. *J. Ethnopharmacol.*, 111: 504-511.
- Lin CL, Wang CC, Chang SC, Inbaraj BS, Chen BH (2009). Antioxidative activity of polysaccharide fractions isolated from *Lycium barbarum* Linnaeus. *Int. J. Biol. Macromol.*, 45: 146-151.
- Luo Q, Cai Y, Yan J, Sun M, Corke H (2004). Hypoglycemic and hypolipidemic effects and antioxidant activity of fruit extracts from *Lycium barbarum*. *Life Sci.*, 76: 137-149.
- Ma M, Liu GH, Yu ZH, Chen G, Zhang X (2009). Effect of the *Lycium barbarum* polysaccharides administration on blood lipid metabolism and oxidative stress of mice fed high-fat diet in vivo. *Food Chem.*, 113: 872-877.
- Mao F, Xiao B, Jiang Z, Zhao J, Huang X, Guo J (2011). Anticancer effect of *Lycium barbarum* polysaccharides on colon cancer cells involves G0/G1 phase arrest. *Med. Oncol.*, 28: 121-126.
- Morillas-Ruiz J, Zafrilla P, Almar M, Cuevas MJ, López FJ, Abellán P, Villegas JA, González-Gallego J (2005). The effects of an antioxidant-supplemented beverage on exercise-induced oxidative stress: results from a placebo-controlled double-blind study in cyclists. *Eur. J. Appl. Physiol.*, 95: 543-549.
- Motta S, Letellier C, Ropert M, Motta C, Thiébaud JJ (2009). Protecting effect of vitamin E supplementation on submaximal exercise-induced oxidative stress in sedentary dogs as assessed by erythrocyte membrane fluidity and paraoxonase-1 activity. *Vet. J.*, 181: 288-295.
- Ni H, Qing D, Kaisa S, Lu J (2004). The study on the effect of LBP on cleaning hydroxyl free radical by EPR technique. *Zhong Yao Cai*, 27: 599-600.
- Potterat O (2010). Goji (*Lycium barbarum* and *L. chinense*): Phytochemistry, pharmacology and safety in the perspective of traditional uses and recent popularity. *Planta. Med.*, 76: 7-19.
- Powers SK, Jackson MJ (2008). Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. *Physiol. Rev.*, 88(4): 1243-1276.
- Reid MB, Stokić DS, Koch SM, Khawli FA, Leis AA (1994). N-acetylcysteine inhibits muscle fatigue in humans. *J. Clin. Invest.*, 94(6): 2468-2474.
- Sachdev S, Davies KJ (2008). Production, detection, and adaptive responses to free radicals in exercise. *Free Radic. Biol. Med.*, 44(2): 215-223.
- Skarpańska-Stejnborn A, Basta P, Pilaczyńska-Szcześniak Ł, Horoszkiewicz-Hassan M (2010). Black grape extract supplementation attenuates blood oxidative stress in response to acute exercise. *Biol. Sport.*, 27: 41-46.
- Smarsh DN, Liburt N, Streltsova J, McKeever K, Williams CA (2010). Oxidative stress and antioxidant status in intensely exercising horses administered nutraceutical extracts. *Equine. Vet. J.*, 38: 317-322.
- Urso ML, Clarkson PM (2003). Oxidative stress, exercise, and antioxidant supplementation. *Toxicology*, 189(1-2): 41-54.

- Vani M, Reddy GP, Reddy GR, Thyagaraju K, Reddanna P (1990). Glutathione-S-transferase, superoxide dismutase, xanthine oxidase, catalase, glutathione peroxidase and lipid peroxidation in the liver of exercised rats. *Biochem. Int.*, 21(1): 17-26.
- Wang HX, Ng TB (1999). Natural products with hypoglycemic, hypotensive, hypocholesterolemic, antiatherosclerotic and antithrombotic activities. *Life Sci.*, 65: 2663-2677.
- Wang L, Zhang HL, Lu R, Zhou YJ, Ma R, Lv JQ, Li XL, Chen LJ, Yao Z (2008). The decapeptide CMS001 enhances swimming endurance in mice. *Peptides*, 29(7): 1176-1182.
- Wang YW, Fu WZ, Tan ZY (2006). Experimental study on the anti-fatigue action of wolfberry fruit. *Chin. Trop. Med.*, 8: 44-47.
- Yao LQ, Li FL (2010). *Lycium barbarum* polysaccharides ameliorates physical, antiatherosclerotic and antithrombotic activities. *Afr. J. Agric. Res.*, 5: 2153-2157.
- Zhang M, Izumi I, Kagamimori S, Sokejima S, Yamagami T, Liu Z, Qi B (2004). Role of taurine supplementation to prevent exercise-induced oxidative stress in healthy young men. *Amino Acids*, 26: 203-207.
- Zhang QJ, Li QX, Xiong ZY, Zhang QH (2003). Effects of exhaustive exercise on biochemical indexes of endurance-trained mice. *Chin. J. Appl. Phys.*, 19(4): 363-366.
- Zhao R, Li QW, Li J, Zhang T (2009). Protective effect of *Lycium barbarum* polysaccharide 4 on kidneys in streptozotocin-induced diabetic rats. *Can. J. Physiol. Pharmacol.*, 87: 11-19.