

Anti-Fatigue and Anti-Hypoxic Effects of *Lycium barbarum* Polysaccharides

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Abstract. The purpose of the present study was to evaluate the anti-fatigue and anti-hypoxic effects of *Lycium barbarum* polysaccharides (LBP) in mice. The mice were randomly divided into four groups, a control group and three treatment groups. The treated groups received different doses of LBP (125, 250 and 500 mg/kg body) by gavage every day for 28 days, while the control group received distilled water. Forced swimming test and normobaric hypoxia test were conducted on the final day of experimentation. The results showed that LBP could extend the exhaustive swimming time, decrease the blood lactic acid (BLA) and serum urea nitrogen (SUN) levels, and increase liver and muscle glycogen contents in forced swimming test. Meanwhile, LBP could extend hypoxic survival time in normobaric hypoxia test. This indicated that LBP had anti-fatigue and anti-hypoxic effects.

Introduction

Lycium barbarum belonging to the plant family *Solanaceae*. Red-colored fruits of *Lycium barbarum* has been widely used for 2000 years in Traditional Chinese Medicine (TCM) [1]. Several functional components including carotenoids, flavonoids and polysaccharides in *Lycium barbarum* fruits correlated with their bioactivities have been investigated. Polysaccharides are an important functional component of *Lycium barbarum* fruits, and composed of arabinose, glucose, galactose, mannose, xylose and rhamnose [2]. Previous studies have shown that *Lycium barbarum* polysaccharides (LBP) have a wide range of biological and pharmacological properties, such as antioxidant, neuroprotection, anti-aging, anti-diabetes, antitumor, anti-osteoporosis and immunomodulation [3]. However, few studies have examined the anti-fatigue and anti-hypoxic effects of LBP. Therefore, the research presented here was designed to evaluate the effects of LBP on hypoxia ability and physical fatigue in mice.

Materials and Methods

Plant materials. The dried *Lycium barbarum* fruits was purchased from an herb market in Changchun, China. and authenticated by Prof. Zhu Zhu (Jilin Business and Technology College, Changchun, China) according to the identification standard of the eighth edition of Pharmacopeia of People's Republic of China (2005 PPRC). Samples were ground and passed through 100 mesh screen. The powders were sealed in air-tight plastic bags and stored in a desiccator at -20 °C.

Chemicals and reagents. The detection kits for blood lactic acid (BLA), serum urea nitrogen (SUN), liver glycogen and muscle glycogen was purchased from Jiancheng Bioengineering Institute (Nanjing, China). All the other chemicals used were of analytical-reagent grade and MilliQ grade water was used.

Preparation of *Lycium barbarum* polysaccharides. The powders of *Lycium barbarum* was soaked with distilled water at room temperature for 2.5 h. Then, it was boiled in distilled water for 1.5 h two times. After filtration, the combined filtrates were concentrated by a rotavapor at 60 °C, and then precipitated using 95% ethanol, 100% ethanol and acetone, respectively. After filtering, the

precipitate was collected and washed with 95% ethanol, absolute ethyl alcohol and acetone by turns, dried at 50 °C and *Lycium barbarum* polysaccharides (LBP) were obtained [4].

Experimental animals. Male Kunming mice weighing 18 - 22 g were used in this study. The animals were maintained at room temperature (20 - 22 °C) with a 12 h light and dark cycle in the animal house. Food and tap water were available *ad libitum*. This experiment was approved by the Bioethic Committee of the Jilin Business and Technology College, and the procedures of the experiment were strictly according to generally accepted international rules and regulations.

Grouping of animals. Following a week-long adaptive phase, the mice were randomly divided into four groups (n = 16 in each group), a control group and three treatment groups. The first group designated as control group was administered with 2.0 mL distilled water by gavage every day for 28 days. The second, third and fourth group designated as treatment groups were administered with LBP of 125, 250 and 500 mg/kg body weight for 28 days, respectively. LBP was dissolved in 2.0 mL of distilled water.

Forced swimming test. After 28 days, 30 minutes after the last administration, eight mice were taken out from each group for the forced swimming test. The animals were placed in an acrylic plastic pool (80 × 60 × 45cm) filled with 35 cm depth of water. Water was maintained at a constant temperature of 30 ± 1 °C during the swimming protocol. The tail of each mouse was loaded with a tin wire, which was 7 per cent of its body weight. Exhaustion was determined by observing loss of coordinated movements and failure to return to the surface within 10 s [5] After the exhaustive swimming exercise, the mice were killed by cervical dislocation, the blood was collected in sterilized tubes without the anticoagulant for BLA and SUN analysis. Then the livers and gastrocnemius muscles were rapidly excised and frozen in liquid nitrogen for storage at -80°C until required for liver and muscle glycogen analysis.

Normobaric hypoxia test. After 28 days, 30 minutes after the last administration, remaining eight mice were taken out from each group for the normobaric hypoxia test. single mouse input in the grinding jar (250 mL, 25 g sodium lime then placed in), and the bottle was closed immediately [6]. The hypoxic survival time was recorded.

Statistical analysis. All values are presented as means ± standard deviations (SD). Statistical comparisons were compared by one-way analysis of variance (ANOVA). The results were considered statistically significant if the p values were 0.05 or less.

Results and Discussion

Effects of LBP on exhaustive swimming time of mice. Forced swimming test has been used extensively for the evaluation of anti-fatigue effects of novel compounds, since it can reflect the fatigue degree of movement and objectively the physical ability of the body [7]. During exhaustive exercise, oxygen consumption increased suddenly in muscle, which might be the etiology of fatigue. As shown in Fig. 1, exhaustive swimming time in the second, third and fourth groups were significantly longer compared with that in the first group (p<0.05). The results indicated that LBP had anti-fatigue effects.

Effects of LBP on blood lactic acid of mice. Blood lactic acid (BLA) is the glycolysis product of carbohydrate under an anaerobic condition, and glycolysis is the main energy source for intense exercise in a short time. With the accumulation of BLA, the pH in muscle tissue and blood reduces, which is harmful to some organs and also causes fatigue [8]. As shown in Fig. 2, the BLA levels in the second, third and fourth groups were significantly lower compared with that in the first group (p<0.05). The results indicated that LBP could quickly remove the BLA and postpone the appearance of fatigue.

Effects of LBP on serum urea nitrogen of mice. Serum urea nitrogen (SUN), the metabolic outcome of protein and amino acid, was a sensitive index to evaluate the bearing capability when body suffered from a physical load. Protein and amino acids have a stronger catabolic metabolism when the body cannot obtain enough energy from sugar and fat catabolic metabolism[9]. There was a positive correlation between urea nitrogen in vivo and exercise tolerance. As shown in Fig. 3, the

SUN levels in the third and fourth groups were significantly lower compared with that in the first group ($p < 0.05$). Although SUN levels in the second group were also decreased, no significant difference was observed ($p > 0.05$). The results indicated that LBP might reduce catabolic decomposition of protein for energy.

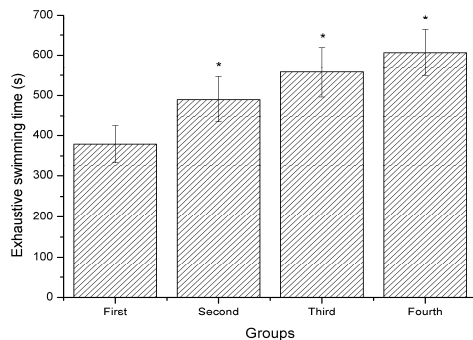


Fig. 1. Effects of LBP on exhaustive swimming time of mice. $p < 0.05$ when compared to the first group

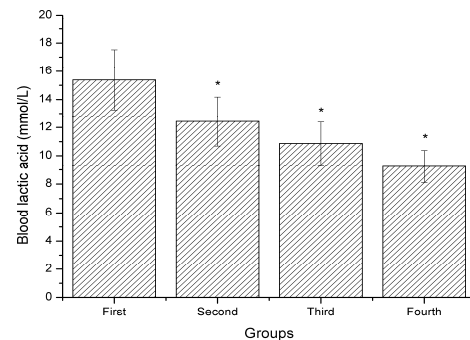


Fig. 2. Effects of LBP on blood lactic acid of mice. $p < 0.05$ when compared to the first group

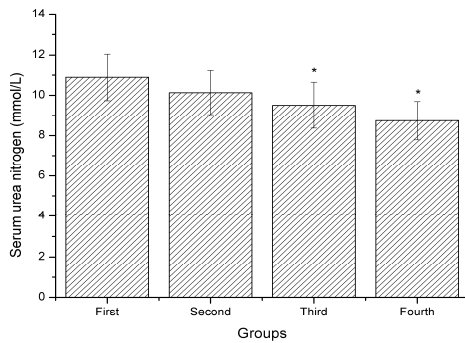


Fig. 3. Effects of LBP on serum urea nitrogen of mice. $p < 0.05$ when compared to the first group

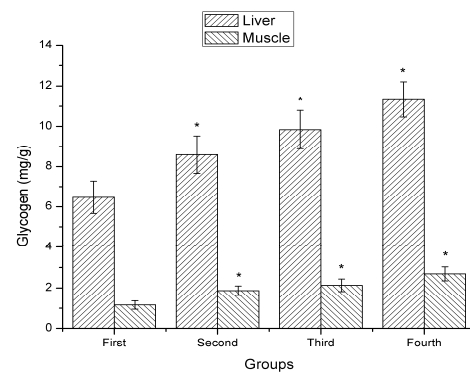


Fig. 4. Effects of LBP on liver and muscle glycogen of mice. $p < 0.05$ when compared to the first group

Effects of LBP on liver and muscle glycogen of mice. Glycogen is regarded as an important source of energy during exercise. Energy for exercise is derived initially from the breakdown of glycogen in muscle, after strenuous exercise may be depleted and at later stages the energy will be derived from liver glycogen [10]. Increase in glycogen consumption has been related to elevated fatigue. As shown in Fig. 4, the liver glycogen contents in the second, third and fourth groups were significantly higher compared with that in the first group ($p < 0.05$). The muscle glycogen contents in the second, third and fourth groups were significantly higher compared with that in the first group ($p < 0.05$). The results indicated that LBP might regulate or maintain normality of organic glycogen levels. This may be one of the mechanisms of its anti-fatigue effects.

Effects of LBP on hypoxic survival time of mice. The animal model of normobaric hypoxia is commonly used in evaluating or screening for novel compounds with anti-hypoxic effects. The survival time of animals in a sealed container directly reflects the anti-hypoxic effects. Many studies have pointed out that exercise endurance is closely related to anti-hypoxic ability in living body [15]. The longer anti-hypoxic time leads to the lower oxygen consumption, which reflects higher utilization of oxygen, helps save energy during exercise, and improves exercise endurance. As shown in Fig. 5, the hypoxic survival time in the second, third and fourth groups were significantly longer compared with that in the first group ($p < 0.05$). The results indicated that LBP had anti-hypoxic effects, and it is beneficial in relieving fatigue.

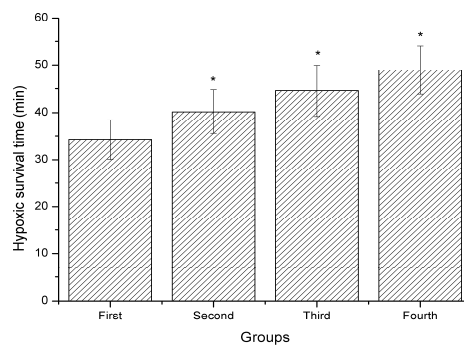


Fig. 5. Effects of LBP on hypoxic survival time of mice. $p < 0.05$ when compared to the first group

Summary

The present study demonstrates that LBP had anti-fatigue and anti-hypoxic effects. LBP could extend the exhaustive swimming time, decrease the BLA and SUN levels, and increase liver and muscle glycogen contents in forced swimming test. Meanwhile, LBP could extend hypoxic survival time in normobaric hypoxia test.

Acknowledgements

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