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Full Length Research Paper

Lycium barbarum polysaccharides ameliorates physical fatigue

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This study was designed to determine the effect of *Lycium barbarum* polysaccharides (LBP) on physical fatigue in mice. 96 male Balb/c mice were divided randomly into four groups based on body weight. One of the group was the control group, the others were LBP treated groups (100, 200, 400 mg/kg body weight). Forced swimming test of mice were carried out after 28 days of LBP administration, and the blood lactate and tissue glycogen contents were determined. Results showed that LBP can extend the swimming time of the mice, effectively delay the increasing of lactate in the blood, and promotes sparing of glycogen. It suggested that LBP contributes to enhancement of physical strength and ameliorates physical fatigue.

Key words: *Lycium barbarum* polysaccharides, physical fatigue, mice.

INTRODUCTION

Lycium barbarum belongs to the plant family solanaceae. It has been commonly used as traditional Chinese medicine and herbal foods for health promotion in China for 2300 years. Moreover, It has attracted tremendous attention as a potentially important agricultural resource, and has been widely applied in the fields of agriculture, medicine, pharmaceuticals, and functional food in the last 50 years (Kim et al., 1997; Yu et al., 2005; Le et al., 2007; Ma et al., 2009). *L. barbarum* fruit is a famous tradi-tional Chinese herbal medicine which has functions of nourishing the kidney and producing essence, nourishing the liver and brightening the eyes, reducing blood glucose and serum lipids, anti-aging, immuno-modulating and male fertility-facilitating (Peng et al., 2001; Amagase and Nance, 2008; Chang and So, 2008).

The active components of *L. barbarum* fruit primarily contain water-soluble polysaccharides. *L. barbarum* can be extracted with hot water followed by precipitation with ethanol to obtain high quantity of polysaccharides. *L. barbarum* polysaccharides(LBP) are estimated to comprise 5 to 8% of the dried fruit (Chao et al., 2006; Chen and Mu, 2007; Meng et al., 2009). Although numerous studies have demonstrated that LBP possesses anti-oxidative, anti-stress, immuno-modulating, anti-diabetic, anti-aging and antitumor properties (Qi et al., 2001; Wang et al., 2002; Zhang et al., 2005; Wu et al., 2006; Li et al., 2007), to our knowledge, there have been limited studies investigating its effects on physical fatigue. Therefore, in this study, we investigated the effects of LBP on physical fatigue in mice.

MATERIALS AND METHODS

Materials

All reagents used in this study were of analytical grade and com-mercially obtained from the Huzhou Chemical Reagent Co., Ltd. (Huzhou, China). The dried *L. barbarum* fruit were bought from Huzhou Medical Material Corporation, and identified by Professor Wang, Huzhou Teachers College. Voucher specimens (HZ-PLT07096) were preserved in Huzhou Natural Product Research Institute.

Preparation of LBP

Preparation of Lycium barbarum polysaccharides (LBP) was as

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described previously and was modified (Luo et al., 2004; Li et al., 2007; Ma et al., 2009). The dried *L. barbarum* fruit were ground to a fine powder, placed in boiling water and decocted for 2 h. The juice was then centrifuged at 9000 r/min for 20 min to remove the precipitate. 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 polysaccharides. The content of the purified polysaccharides was measured by phenol-sulfuric method (Robyt and Bemis, 1967). Absorbance was measured at 490 nm with standard glucose solutions from 0 to 100 mg/L spectrophotometrically. Result showed that the content of the polysaccharides in the extract may reach 96.27%.

Animals and grouping

Five-week-old, male Balb/c mice were purchased from Huzhou Research Animal Center (Huzhou, China) and maintained under a light cycle (12 h light/dark), temperature (21 - 23°C) and humidity (40 - 60%) conditions. Mice were provided a basal diet and water *ad libitum*. Per 100 mg of basal diet of the subjects included corn starch, 50.00 mg ; casein, 20.00 mg; sucrose,13.00 mg; soybean oil, 7.00 mg; powdered cellulose, 5.00 mg; vitamin mix, 3.7 mg; mineral mix, 1.00 mg; L-Cystine, 0.30 mg. After a 1-week acclimation period, 96 mice were divided randomly into four groups based on body weight (n = 24). One group was the control (C) group; the others were LBP treated groups: low-dose (LD) group, medium-dose (MD) group and high-dose (HD) group.

C group: Mice in control group were orally administrated with the volume (0.03 mL) of physiological saline for 28 consecutive days.
LD group: Mice of low-dose group were treated by oral administration with the same volume of LBP at a dose of 100 mg/kg body weight/day dissolved in physiological saline for that same period.
MD group: Mice of medium-dose group were treated by oral administration with the same volume of LBP at a dose of 200 mg/kg body weight/day dissolved in physiological saline for that same period.
HD group: Mice of high-dose group were treated by oral administration with the same volume of LBP at a dose of 400 mg/kg body weight/day dissolved in physiological saline for that same period.

The doses used in this study were confirmed to be suitable and effective in tested mice according to preliminary experiments.

Forced swimming test

We used forced swimming test to evaluate the effect of LBP on physical fatigue. The apparatus used in this test was an acrylic plastic pool (90 × 45 × 45 cm) filled with water maintained at 30±1℃.The water in the acrylic plastic pool was 35 cm deep. Eight mice were taken out from each group to make forced swimming test after being administrated with different dose of LBP for 28 days. Mice had a load attached (5% body weight) to the tail for the duration of the swim-to exhaustion exercise. The mice were assessed to be fatigued when they failed to rise to the surface of the water to breathe within 5 s (Kamakura et al., 2001; Ikeuchi et al., 2006). The time of each group of mice was averaged and the data of the different groups was analyzed.

Analysis of blood lactate contents

Eight mice were taken out from each group for blood lactate analyses after being administrated with different dose of LBP for 28 days. Mice were forced to swim for 30 min after weight loading (2% body weight). Twenty microliter of blood was collected from the veins of the tails of mice after the last administration of LBP. Another twenty microliter of blood samples was collected immediately after mice have been swimming. Then blood lactate was tested according to the recommended procedures provided by the commercial diagnostic kit from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Analysis of tissue glycogen contents

Eight mice were taken out from each group for tissue glycogen analyses after being administrated with different dose of LBP for 28 days. Mice were forced to swim 90 min without a load. After an hour's rest, mice was anesthetized to death with high concentration aether in an acrylic plastic immobilizer and its liver and gastrocnemius muscle were quickly dissected out, frozen in liquid nitrogen, and kept at -70°C until analysis for glycogen contents. Then tissue glycogen was tested according to the recommended procedures provided by the commercial diagnostic kit from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Statistical analyses

All data in table are expressed as mean±SD 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).

RESULTS AND DISCUSSION

Effect of LBP administration on the body weight of mice

Figure 1 showed the body weight change of the mice during the experimental period. The weights of the mice were measured after they were administrated with different dosages of LBP for 28 days. Results showed that the increased weights in the experimental groups were of no significant difference compared with the control group ($P > 0.05$). So the LBP had no significant effect on body weight.

Effect of LBP on the swimming time to exhaustion of mice

Effect of LBP on physical fatigue was evaluated in a forced swimming test. As shown in Figure 2, the swimming time to exhaustion of the treated groups (LD, MD and HD group) were significantly longer than that of control group (C group) ($P < 0.05$).

The forced swimming test has been used to evaluate

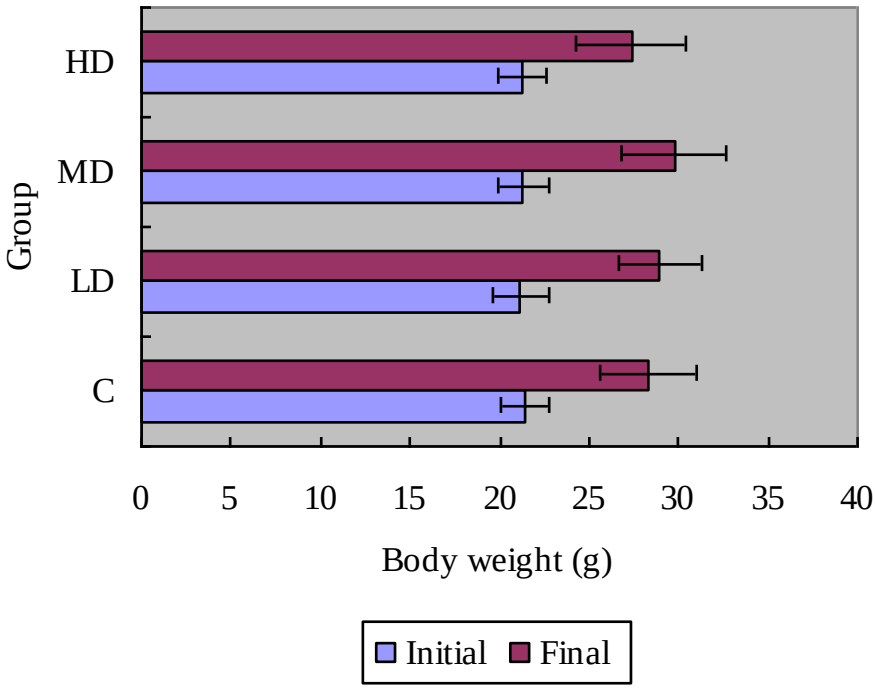

Figure 1. Effect of LBP administration on body weight of mice (mean±SD, n = 24, g).

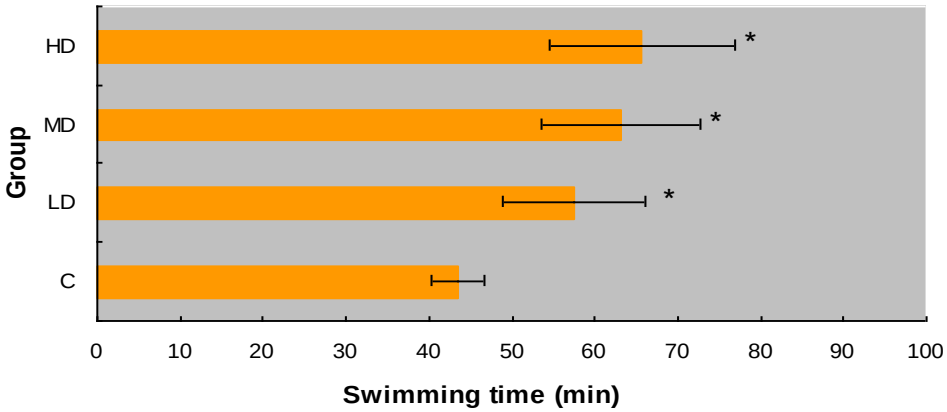

Figure 2. Effect of LBP on the swimming time to exhaustion of mice. *P < 0.05 vs C group.

the anti-fatigue effects of various compounds (Koh et al., 2003; Murase et al., 2006; Nozawa et al., 2009). It is commonly accepted that swimming is an experimental exercise model (Jung et al., 2004). In this study, the data showed that oral administration of LBP can evidently extend mice's swimming time (Ji et al., 1996), which indicated that LBP can enhance the swimming capacity by lessening of fatigue in mice.

Effect of LBP on the blood lactate contents of mice

Blood lactate contents of mice are represented in Table 1. There was no significant change in the blood lactate contents among all the groups before the swimming exercise. After swimming, blood lactate contents of the treated groups (LD, MD and HD group) were significantly lower than that of control group (C group) ($P < 0.05$).

Lactate (2-hydroxypropanoic acid) was considered a metabolic end product of glycolysis and a potential candidate for inducing fatigue (Morihara et al., 2006; Zhang et al., 2009). Some studies (Chase and Bemis, 1988; Murase et al., 2006; Yan et al., 2008) indicated that when lactate builds up in myocytes, intracellular pH drops, contributing to the onset of fatigue. In this study, the data showed that oral administration of LBP can

Table 1. Effect of LBP on the blood lactate contents of mice (mean±SD, n = 8, mmol/L).

Group	Before exercise	After exercise
C	4.28±0.45	12.61±1.36
LD	4.36±0.29	8.94±1.24*
MD	4.24±0.39	8.11±1.47*
HD	4.33±0.52	8.52±1.51*

*P < 0.05 vs C group.

Table 2. Effect of LBP on the tissue glycogen contents of mice (mean±SD, n = 8, mg/g).

Group	Liver glycogen	Muscle glycogen
C	7.93±3.11	1.17±0.34
LD	10.87±4.15*	1.92±0.63*
MD	14.85±4.53*	2.16±0.57*
HD	15.21±5.32*	2.03±0.48*

*P < 0.05 vs C group.

effectively delay the increase of lactate in the blood and postpone the appearance of physical fatigue.

Effect of LBP on the tissue glycogen contents of mice

Tissue glycogen contents are shown in Table 2. After swimming, liver and gastrocnemius muscle glycogen contents of the treated groups (LD, MD and HD group) were significantly higher than that of control group (C group) (P < 0.05).

In this study, the data showed that oral administration of LBP can promote sparing of glycogen. The glycogen-sparing effect of LBP can provide an important survival advantage in situations requiring extended periods of prolonged endurance exercise because glycogen depletion is associated with physical exhaustion, and slower utilization of glycogen results in improved endurance exercise performance.

As one of the sources of blood glucose, glycogen plays an important role in controlling the availability of cellular energy (Ikeuchi et al., 2006). It is possible that LBP may have promoted glycogenolysis restraint or gluconeogenesis.

In conclusion, our data suggest that LBP can extend the swimming time of the mice, effectively delay the increasing of lactate in the blood, and promotes sparing of glycogen, suggesting that LBP contributes to enhancement of physical strength and ameliorates physical fatigue. Further work to elucidate the mechanism by which LBP promotes sparing of glycogen will be clearly warranted.

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