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Full Length Research Paper

Fatigue-alleviating effect of polysaccharides from *Cyclocarya paliurus* (Batal) Iljinskaja in mice

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In this study, the fatigue-alleviating effect of polysaccharides from *Cyclocarya paliurus* (Batal) Iljinskaja (PCP) in mice were evaluated using a weight-loaded swimming test and some biochemical parameters related to fatigue, including serum urea nitrogen (SUN), blood lactic acid (BLA), hemoglobin (Hb) and hepatic glycogen were measured. Male Kunming mice were administered PCP at doses of 0, 25, 50 and 100 mg/kg for 4 weeks. The results showed that PCP can increase swimming time to exhaustion, Hb and hepatic glycogen contents whilst reducing SUN and BLA contents, indicating an alleviating effect on exercise-induced fatigue in mice.

Key words: Polysaccharides, *Cyclocarya paliurus* (Batal) Iljinskaja, fatigue, swimming test, mice.

INTRODUCTION

Cyclocarya paliurus (Batal.) Iljinskaja, a Chinese native plant, belongs to the genus *Cyclocarya* Iljinskaja (Juglangdaceae) (Yi et al., 2002; Xie et al., 2010a). This plant is grown on cloudy and foggy highlands in southern China including Anhui, Fujian, Hubei, Hunan, Jiangsu, Jiangxi, Sichuan, Guizhou, and Zhejiang Provinces. And it is commonly called the 'sweet tea tree' because of the flavour of its leaves (Xie et al., 2010b). The leaves of *C. paliurus* have been a food resource for maritime people for a long time, and have also been used for drug formulations in traditional Chinese medicine (TCM), as well as for an ingredient in functional foods in China (Li et al., 2002; Birari and Bhutani, 2007; Fang et al., 2011). Significant attention has recently been drawn to the use of *C. paliurus* for developing functional food, as *C. paliurus* produces a great variety of nutrients that are essential for human health. *C. paliurus* health tea, the aqueous extract of *C. paliurus* leaves, is already known as a functional health food for ailments, the enhancement of mental efficiency, and recovery from mental fatigue, has been become the first Food and Drug Administration (FDA)-approved health tea of China in 1999 (Xu and Song, 2004; Xie et al., 2010a). Recently, the wide array of therapeutic effects of *C. paliurus* have been reported, such as enhancement of mental efficiency and hypolipidaemic, antihypertensive and immunomodulatory effects (Kurihara et al., 2003; Li et al., 2008, 2011).

Recently, the bio-activities of polysaccharides from plants and fungi have attracted more and more attention in biochemistry and medicine. In the last few decades, polysaccharides from plants and fungi exhibit varied bio-activities such as antioxidant, antidiabetic, antitumor, anticancer, antifatigue, antiviral, antibacterial, antifungal, anticoagulant and immunological activities (Hwang et al., 2005; Yu et al., 2006; Kardosová and Machová, 2006; Lee et al., 2007; Thierbach and Steinberg, 2009). To date, most studies on *C. paliurus* were concerned about the extract bio-activities, and low molecular weight substances, such as triterpenoids, flavonoids, steroids, saponins and other compounds present in this plant (Shu et al., 1995; Jiang et al., 2006; Wang and Cao, 2007; Fang et al., 2011). Whereas, there have been only a few reports on polysaccharides from *C. paliurus* (PCP) and few on its bio-activities. For example, Liu et al. (2007) found that PCP has anti-tumor activity and can significantly inhibit the proliferation of cervical cancer HeLa cells. Xie et al. (2010) reported that PCP exerted significant scavenging effects on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. Shangguan et al. (2010) found that PCP possesses a hypoglycemic effect in alloxan-induced hyperglycemic mice. To the best of our

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knowledge, there are no previous reports on the effect of the anti-fatigue. In this study, we investigated whether PCP can improve exercise-induced fatigue. In order to assess potential mechanisms of PCP bio-activities, we measured some biochemical parameters related to fatigue, including serum urea nitrogen (SUN), blood lactic acid (BLA), hemoglobin (Hb) and hepatic glycogen.

MATERIALS AND METHODS

Plant material

The dried leaves of *C. paliurus* were provided by Zhangjiajie Hongmao ecological Co. (Hunan, China). A voucher specimen (registration number: 2010069) has been deposited in the Natural Products Laboratory, Zhengzhou University. All samples were sliced and ground into fine powder in a mill before extraction.

Extraction of PCP

The method of Xie et al. (2007) was used in the extraction of PCP. The procedures are described as follows: the dried leaves of *C. paliurus* powder (100 g) were first weighed and extracted with 1000 ml of 80% ethanol for 24 h to remove the interfering components in the samples at 80°C. The extraction procedure was carried out in the water bath. After filtration, the residue were dried at room temperature and placed in an extraction tube, then extracted twice with ultra-pure water (20:1 weight/volume ratio) at 80°C for 2 h. The extracts were filtered, while warm, through glass wool and centrifuged for 15 min to separate the supernatant and the residue. The Sevag method was used to remove protein components (Staub, 1965). After removing the Sevag reagent, the water phase were concentrated under reduced pressure at 55°C and precipitated with four volumes of ethanol, then kept at 4°C overnight in refrigerator to precipitate polysaccharides. The precipitates formed in the solution were collected and then redissolved in ultra-pure water, centrifuged for 15 min. The supernatant was further dialysed for 36 h in natural water and 12 h in ultra-pure water before concentration under vacuum evaporator at 55°C. Lastly, the precipitate was frozen at -40°C overnight and lyophilized in vacuum freeze dryer. The crude PCP was obtained. The polysaccharide content was measured by the sulfuric acid/phenol method. Briefly, polysaccharides were hydrolyzed to sugar aldehyde in the presence of sulfuric acid and condensed with phenol to give a colored complex, which can be quantified by spectrometry at 480 nm. Then the extraction yield of PCP was 6.27 wt % (dry basis)

Animals

The study protocol was approved by the Institutional Animal Care and Use Committee of Zhengzhou University (Zhengzhou, China.). Healthy male Kunming mice were obtained from Laboratory Animal Center, Medical College of Zhengzhou University. Mice were housed in environmentally controlled conditions (temperature 20±2°C; relative humidity 50 to 60%) with a 12 h light/dark cycle. All animals had free access to standard rodent pellet food and water *ad-libitum*. Animals weighing 18 to 22 g were used in the study.

Experimental design

After an adaptation period for a week, the 64 mice were randomly divided into four groups, with 16 mice in each group. PCP was given to the mice at doses of 0, 25, 50 and 100 mg/kg and the four groups were accordingly named as the control (C) group, PCP low-dose treatment (PL) group, PCP middle-dose treatment (PM) group and PCP high-dose treatment (PH) group. PCP was dissolved in 1 ml of distilled water and the same volume of distilled water was given to mice in C group. Samples were orally administered (8: 00 am) into mice using a feeding atraumatic needle once per day for 4 weeks. The doses of PCP used in this study were confirmed to be suitable and effective in tested rabbits according to our preliminary experiment. The mice were made to swim for 15 min three times a week to accustom them to swimming. After 4 weeks, 8 mice were taken out from each group for weight-loaded swimming test. The other 8 mice were taken out from each group for analyses of some biochemical parameters related to fatigue.

Weight-loaded swimming test

The weight-loaded swimming test was employed in this study to evaluate the effects of PCP on exercise-induced fatigue. The test was induced by forcing animals to swim until exhaustion as described in the literature (Tang et al., 2008). Briefly, 30 min after the last administration, the mice were dropped individually into an acrylic plastic pool (90×45×45 cm) filled with fresh water maintained at 30±1°C, approximately 35 cm deep so that mice could not support themselves by touching the bottom with their tails. A lead block (5% of body weight) was loaded on the tail root of the mice. Mice were regarded as exhaustion when they were underwater for 8 s (Chi et al., 2008), and their swimming time was immediately recorded.

Biochemical analysis

In order to explore the mechanism, some biochemical parameters related to fatigue, including SUN, BLA, Hb and hepatic glycogen were measured. Briefly, 30 min after the last administration, the mice were forced to swim for 90 min without a load. Rested for 60 min, the mice were anesthetized with ether and blood samples were collected in tubes by heart puncture to determine the contents of SUN, BLA and Hb. In addition, immediately after the blood had been collected, the liver was dissected out quickly from the mice, washed with physiological saline and dried with absorbent paper. Then the contents of hepatic glycogen were determined.

Data analysis

Data were expressed as the mean ± SD and analyzed by one-way analysis of variance (ANOVA), followed by Post hoc test (SPSS 15.0). The difference was considered significant when $P < 0.05$.

RESULTS AND DISCUSSION

Effects of PCP on weight-loaded swimming test of mice

The direct appearance of anti-fatigue effect is the elevation of exercise tolerance. Reduced susceptibility to fatigue was interpreted from a longer swim time. A weight-loaded 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

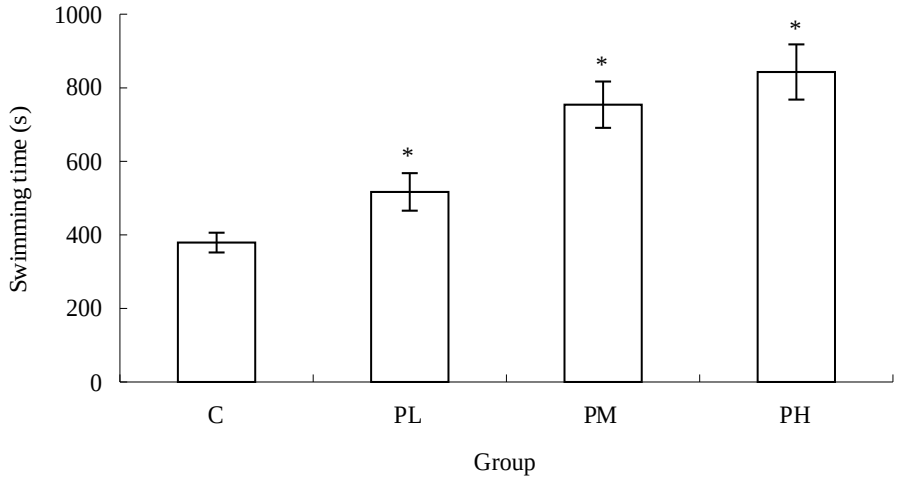

Figure 1. Effects of PCP on weight-loaded swimming test of mice. C group, control group (the mice were administered distilled water); PL group, PCP low-dose treatment group (the mice were administered 25 mg/kg of PCP); PM group, PCP middle-dose treatment group (the mice were administered 50 mg/kg of PCP); PH group, PCP high-dose treatment group (the mice were administered 100 mg/kg of PCP). Values are the means ± S.D.*, $P < 0.05$, compared with control group.

safely (Wang et al., 2008). In the present study, the mice had a weight attached 5% body weigh in the duration of the swim-to-exhaustion. As shown in Figure 1, the swimming time to exhaustion of the PL, PM and PH groups were significantly prolonged compared with that in the C group ($P<0.05$), which is 1.36, 1.99 and 2.23 times longer that in the C group, respectively. These results indicated that PCP can alleviate exercise-induced fatigue. To explore the mechanism, some biochemical parameters related to fatigue were measured.

Effects of PCP on SUN of mice

SUN is one of blood biochemical parameters related to fatigue. Urea nitrogen is the metabolism outcome of protein and amino acid. Urea is formed in the liver as the end product of protein-metabolism and is carried by the blood to the kidneys for excretion (Wang et al., 2006; Ding et al., 2011). Dynamophore in sports include sugar, fat and protein. When movement time not exceed 30 min protein seldom participate in energize and SUN change a little. Protein and amino acids have a stronger katabolic metabolism when body cannot obtain enough energy by sugar and fat catabolic metabolism, after a long time of movement, urea nitrogen obviously increase at this time (Wang et al., 2008). As shown in Figure 2, the SUN contents of the PL, PM and PH groups were significantly decreased compared with that in the control group ($P<0.05$), which is 1.15, 1.21 and 1.34 times lower that in the control group, respectively. These results indicated that decrease in the contents of SUN may be one of the pathways of PCP alleviating exercise-induced fatigue.

Effects of PCP on BLA of mice

BLA content is one of fatigue-relevant factors measured before and after exercise (Watts et al., 1996; Khanna and Manna, 2005; Zheng et al., 2010). Many organs, especially the liver and skeletal muscle, help remove lactic acid from the blood (Lee et al., 2011). However, intense exercise can increase lactic acid production to a point that exceeds the rate of lactic acid removal, this condition resulting in fatigue (Lambert and Flynn, 2002). As shown in Figure 3, the BLA contents of the PL, PM and PH groups were significantly decreased compared with that in the C group ($P<0.05$), which is 1.24, 1.36 and 1.47 times lower that in the C group, respectively. These results indicated that the PCP treated mice experienced a reduction in lactic acid production and/or an increased rate of lactic acid removal, which may be another pathway of PCP alleviating exercise-induced fatigue.

Effects of PCP on Hb of mice

Hb is one of the indicators that reflect the degree of recovery from fatigue after exercise. Its main function is to serve as the carrier for the erythrocyte to transport oxygen and carbon dioxide (Nikinmaa, 1997; Gao and Wu, 2008). Apart from maintaining the acid-alkali balance, the body fluid acid-base balance is very important because a small change can produce major disturbance since it can affect the electrolytes and functionality of enzymes. Earlier studies had reported that higher level of Hb is helpful to improve the exercise ability (Gao and Wu, 2008). Similar results also were observed

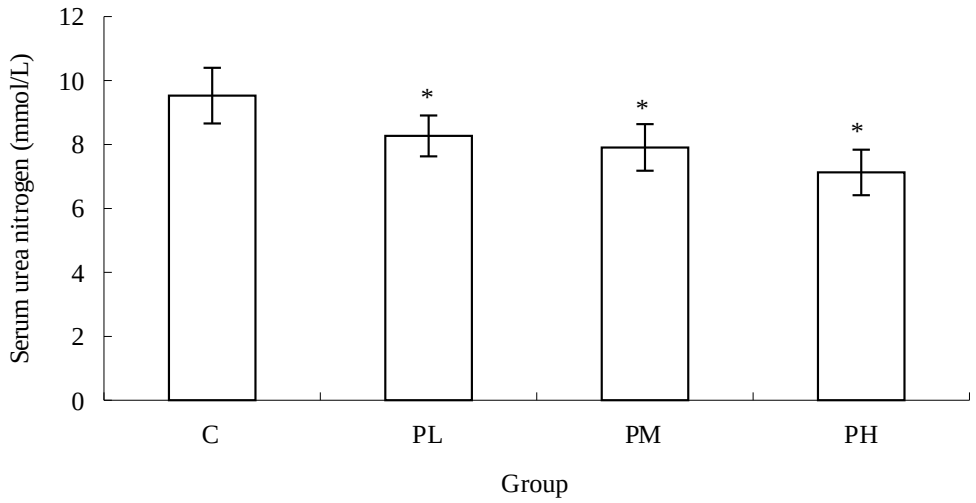

Figure 2. Effects of PCP on serum urea nitrogen of mice. C group, control group (the mice were administered distilled water); PL group, PCP low-dose treatment group (the mice were administered 25 mg/kg of PCP); PM group, PCP middle-dose treatment group (the mice were administered 50 mg/kg of PCP). PH group, PCP high-dose treatment group (the mice were administered 100 mg/kg of PCP). Values are the means ± S.D. *, $P < 0.05$, compared with control group.

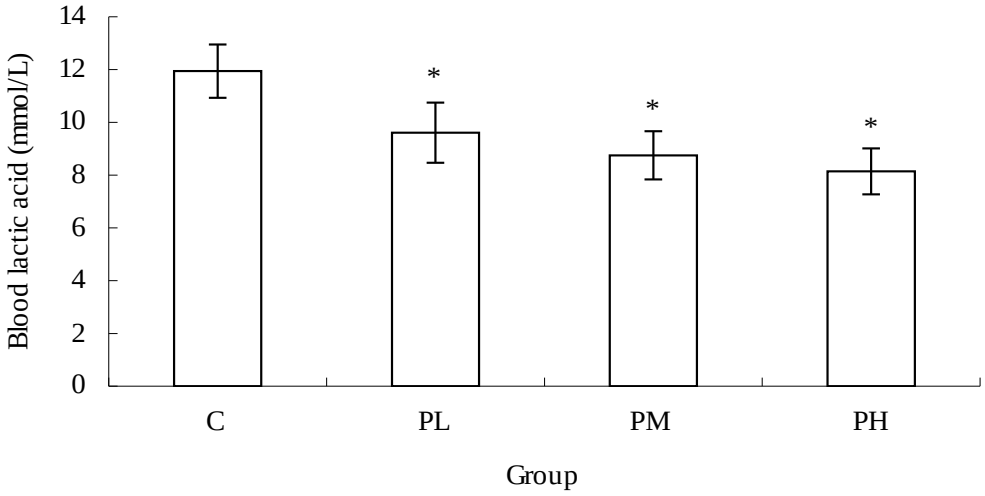

Figure 3. Effects of PCP on BLA of mice. C group, Control group (the mice were administered distilled water); PL group, PCP low-dose treatment group (the mice were administered 25 mg/kg of PCP); PM group, PCP middle-dose treatment group (the mice were administered 50 mg/kg of PCP); PH group, PCP high-dose treatment group (the mice were administered 100 mg/kg of PCP). Values are the means ± S.D. *$P < 0.05$, compared with control group.

in the present study. As shown in Figure 4, the Hb contents of the PM and PH groups were much higher than that in C group ($P<0.05$). Although Hb contents of the PL group were also increased, no significant difference was observed ($P>0.05$). These results indicated that increase in the contents of Hb may be another pathway of PCP alleviating exercise-induced fatigue.

Effects of PCP on hepatic glycogen of mice

It is generally accepted that endurance capacity decreases if the available energy is exhausted. Glycogen is the major energy source during exercise; the increase in glycogen stored in liver is an advantage to enhance the physical endurance (Wagenmakers et al., 1991). Depletion of hepatic glycogen is an important factor in the

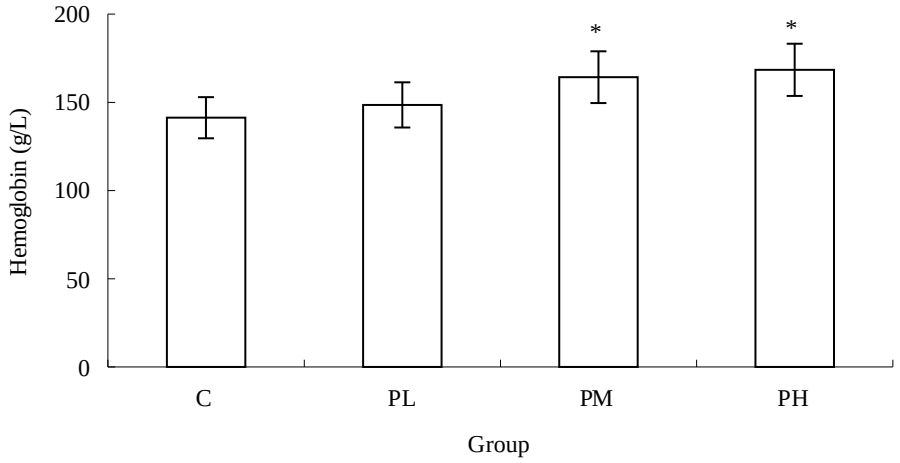

Figure 4. Effects of PCP on Hb of mice. C group, Control group (the mice were administered distilled water); PL group, PCP low-dose treatment group (the mice were administered 25 mg/kg of PCP); PM group, PCP middle-dose treatment group (the mice were administered 50 mg/kg of PCP); PH group, PCP high-dose treatment group (the mice were administered 100 mg/kg of PCP). Values are the means ± S.D. *P < 0.05, compared with control group.

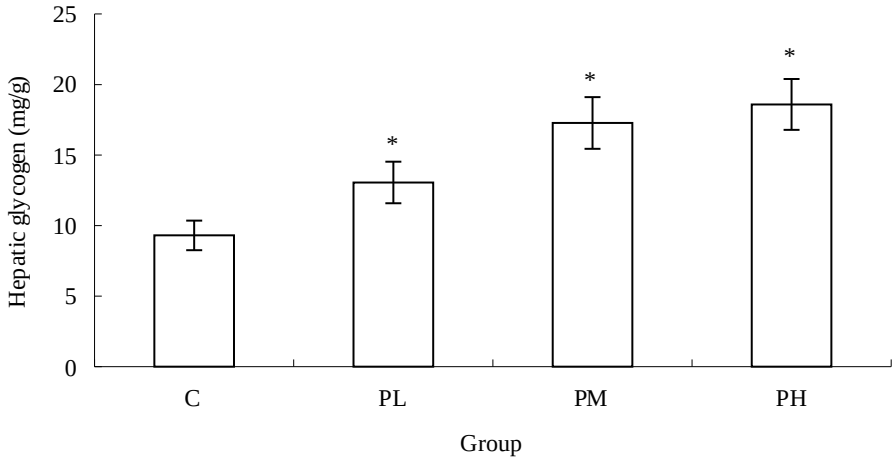

Figure 5. Effects of PCP on hepatic glycogen of mice. C group, Control group (the mice were administered distilled water); PL group, PCP low-dose treatment group (the mice were administered 25 mg/kg of PCP); PM group, PCP middle-dose treatment group (the mice were administered 50 mg/kg of PCP); PH group, PCP high-dose treatment group (the mice were administered 100 mg/kg of PCP). Values are the means ± S.D. *P < 0.05, compared with control group.

exercised fatigue, which may lead to hypoglycemia impairing nervous function (Dohm et al., 1983). The previous studies have indicated that glycogen accumulates in the body and delays fatigue after exercise. As shown in Figure 5, the hepatic glycogen contents of the PL, PM and PH groups were significantly increased compared with that in the C group (P<0.05), which is 1.40, 1.86 and 1.99 times higher that in the C group, respectively. These results indicated that increase in the contents of hepatic glycogen may be another pathway of PCP alleviated exercise-induced fatigue.

Conclusions

The present study demonstrated that PCP can increase swimming time to exhaustion, Hb and hepatic glycogen contents whilst reducing SUN and BLA contents indicate an alleviating effect on exercise-induced fatigue in mice. However, further research needs to be carried out to

evaluate its anti-fatigue effect at cellular and molecular levels.

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