

Full Length Research Paper

Effects of crude polysaccharides from *Purslane* on fatigue induced by forced swimming

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The objective of this study was to investigate the effects of crude polysaccharides from *Purslane* (PFP) supplementation on fatigue induced by forced swimming in mice. 96 mice were divided randomly into four groups: Control group (CG), low-dose PFP supplemented group (PFP-LG), middle-dose PFP supplemented group (PFP-MG) and high-dose PFP supplemented group (PFP-HG). Each group contains 24 animals. All were administered orally and daily for 4 weeks. Control group received isotonic saline solution (50 ml/kg bodyweight); and supplemented groups orally obtained 100, 200 and 400 mg/kg bodyweight of PFP in appropriate volumes of physiological saline. Changes of the body weights of the mice were observed during initial and terminal stages of the experiment along with the swimming capacity and corresponding biochemical parameters including blood lactic acid (BLA), serum urea nitrogen (SUN) and hepatic glycogen. The results of this study showed that PFP supplementation could extend the swimming time to exhaustion of the mice, as well as increase the hepatic glycogen contents, while decreasing the BLA and SUN contents. These indicated that PFP could alleviate fatigue induced by forced swimming in mice.

Key words: Crude polysaccharides from *Purslane*, fatigue, forced swimming, mice.

INTRODUCTION

Fatigue is an everyday phenomenon, which we all experience. Yet, it is difficult to define fatigue since it is not a uniform phenomenon and has various appreciations depending on the context. When it is used in exercise

induced decrease of the maximal muscular force physiology, we usually think of fatigue as an exercise- (Gandevia, 2001; Taylor et al., 2006; Scheidegger et al., 2010). Many reports have indicated that exercise-induced fatigue can be attributed to the following factors. First, myoglobin and an energy metabolic system coenzyme leak out into the blood from cells and tissues damaged by exercise, and destruction of red blood cells occurs. Second, exercise promotes consumption of energy sources, such as glycogen, by mobilizing internal energy metabolism to the maximum and using and depleting the energy source. Third, through these processes, exercise causes the production and accumulation of products of metabolism, such as lactic acid, in the body (Ikeuchi et al., 2006; Kim et al., 2008; Ding et al., 2010). Therefore,

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Abbreviations: PFP, Polysaccharides from *Purslane*; CG, control group; PFP-LG, low-dose PFP supplemented group; PFP-MG, middle-dose PFP supplemented group; PFP-HG, high-dose PFP supplemented group; BLA, blood lactic acid; SUN, serum urea nitrogen.

recovery from exercise fatigue requires repair of the damage that has occurred in the body. Recently, there has been a great increase in the use of over-the-counter supplements and naturally occurring nutraceuticals for the attenuation of exercise-induced fatigue.

Purslane (*Portulaca oleracea* L.) is a common, herbaceous succulent annual weed, which is distributed extensively in temperate and tropical regions worldwide (Zhu et al., 2010). It has a long history of being used as a medicinal and edible plant in China (Rasheed et al., 2004; Lou et al., 2011). In traditional Chinese medicine, *Purslane* is utilized as an antipyretic, anti-scorbutic, antiseptic, antispasmodic, diuretic, antihelminthic and for treatment of urinary disorders. The aerial parts of the plant are used medicinally for reducing pain and swelling (Xin et al., 2008; Karimi et al., 2010). Recent pharmacological studies have shown muscle relaxant activity, reduction in locomotor activity, increased in the onset time of pentylenetetrazole-induced convulsion, analgesic, anti-inflammatory effects and antioxidant properties (Radhakrishnan et al., 2001; YouGuo et al., 2009; Karimi et al., 2010). *Purslane* contains several biologically active compounds including organic acids, alkaloids, coumarins, flavonoids, cardiac glycosides and polysaccharides etc. (Rasheed et al., 2004; Yang et al., 2007). In recent years, crude polysaccharides isolated from *Purslane* (PFP) are used to treat burns, headaches, stomach, intestinal and liver ailments, cough, shortness of breath and arthritis, for its strong antioxidant, anticancer, anti-microbial, anti-diabetic, antiviral and anti-inflammatory properties (Li et al., 2009; Chen et al., 2010; Dong et al., 2010). However, as far as we know, the anti-fatigue activity of PFP is still poorly understood. Therefore, the present study was to investigate the effects of PFP on fatigue induced by forced swimming.

MATERIALS AND METHODS

Sample collection

Purslane was collected in its fresh state at a local farm in Shanghai city and authenticated by Dr. D. M. Li (Shanghai Botany Institute, China). A voucher specimen of the plant materials (voucher No. KPT-762) was deposited in herbarium of Donghua University (Shanghai, China). Fresh *Purslane* was washed with distilled water and dried with air. The dried *Purslane* was crushed into powder by a plant grinder. The powder of the samples was kept in an airtight container.

Experiment animal

All animal (used in this experiment) handling procedures were performed in strict accordance with the P.R. China legislation, the use and care of laboratory animals, with the guidelines established by Institute for Experimental Animals of Shanghai, and were approved by the Animal Care and Use Committee of the Donghua University (Shanghai, China) for animal experiments. Male Kunming mice weighing 18 to 22 g were obtained from Shanghai Slack Laboratory Animal Co., Ltd. (Shanghai, China). To avoid possible individual differences, only male mice were studied in this study.

Because male animals have small individual differences and there is no obvious physical characteristics when compared with female animals. Animals were acclimatized for 1 week before being used for the experiment. Before and during the experiment the mice were housed under controlled environmental conditions of temperature ($22 \pm 2^\circ\text{C}$) and a 12 h light and dark cycle, and maintained on (unless otherwise stated) standard food pellets and tap water *ad libitum*.

Preparation of polysaccharides from *Purslane*

Purslane powder was extracted in a Soxhlet apparatus with a mixture of chloroform-methanol (2:1, 75°C), and pretreated with 80% ether twice to remove some coloured materials, oligosaccharides, and some small molecule materials. The organic solvent was volatilized and pretreated dry powder was obtained. The pretreated dry powder (500 g) was extracted with boiling water (4000 mL) for 3 h twice. The aqueous extracts combined and centrifuged (2000 g, 20 min), then the supernatant was separated from insoluble residue with nylon cloth (pore diameter: 38 μm). The aqueous extracts were then defatted by the method of Sevag, precipitated by the addition of ethanol to a final concentration of 80% (v/v) and the precipitates were collected by centrifugation (2000 g, 20 min). It was then solubilized in deionized water and lyophilized to get crude polysaccharides from *Purslane* (Li et al., 2009; Lou et al., 2011). The contents of polysaccharides were measured by the phenol-sulphuric acid method using glucose as standard.

Experimental design

Mice were trained to accustom themselves to swimming twice (5 min per time) in the first week. During the period, the mice which could not learn to swim were screened out. Then 96 mice were chosen and divided randomly into four groups: control group (CG), low-dose PFP supplemented group (PFP-LG), middle-dose PFP supplemented group (PFP-MG) and high-dose PFP supplemented group (PFP-HG). Each group contains 24 animals. All were administered orally and daily for 4 weeks. Control group received isotonic saline solution (50 ml/kg bodyweight); supplemented groups orally obtained 100, 200 and 400 mg/kg bodyweight of PFP in appropriate volumes of physiological saline. Changes of the body weights of the mice were observed during initial and terminal stages of the experiment along with the swimming capacity and corresponding biochemical parameters including blood lactic acid (BLA), serum urea nitrogen (SUN) and hepatic glycogen.

Forced swimming capacity test

The forced swimming capacity test was employed in this study to evaluate the effects of polysaccharides from *Purslane* on fatigue. After 4 weeks, 12 mice were taken out from each group for the forced swimming capacity test. The procedure used was described previously (Jung et al., 2004; Kamakura et al., 2005) with some modifications. Briefly, 30 min after the last oral administration, the mice were dropped individually into an acrylic plastic pool (90 × 45 × 45 cm) filled with fresh water maintained at $25 \pm 1^\circ\text{C}$, approximately 35 cm deep so that mice could not support themselves by touching the bottom with their tails. A lead block (7% of body weight) was loaded on the tail root of the mice. The swimming time to exhaustion was used as the index of the forced swimming capacity. The mice were assessed to be exhausted when they failed to rise to the surface of water to breathe within a 7 s period (Oh et al., 2003; Jung et al., 2004; Lee et al., 2011).

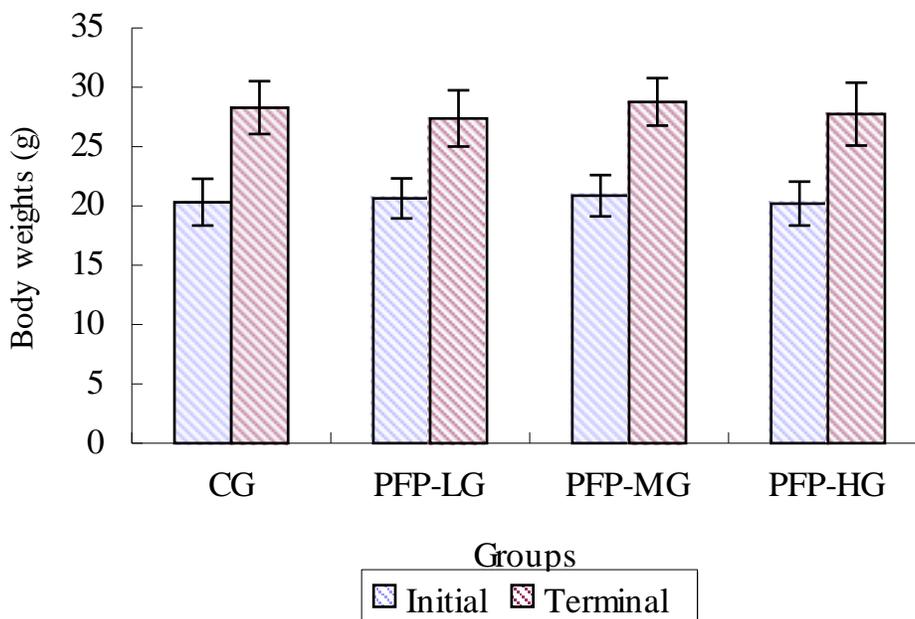


Figure 1. Effect of PFP on body weights of mice.

Determination of some biochemical parameters related to fatigue

After 4 weeks, the other 12 mice were taken out from each group for analyses of some biochemical parameters related to fatigue. 30 min after the last oral administration, the mice were forced to swim in the swimming pool (weight-unloaded) for 90 min. Rested for 60 min, the mice were anesthetized with ether and whole blood samples were collected in tubes by heart puncture. Blood samples were placed for about 1 h at 4°C and centrifuged for 10 min at a speed of 3000 rpm. The supernatant was collected and contents of BLA and SUN were analyzed. 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 content of hepatic glycogen was analyzed. The contents of BLA, SUN and hepatic glycogen were determined by using commercial diagnostic kits (Institute of Biological Engineering of Nanjing Jianchen, Nanjing, China).

Acute toxicity test

Healthy Kunming mice (18 to 22 g) starved overnight were divided into three groups ($n = 8$) and were orally fed with the PFP at 1, 2 and 4 g/kg bodyweight, respectively. The following profiles of animals were observed continuously for 2 h (Li et al., 2009).

Behavioral profile: Alertness, restlessness, irritability, and fearfulness; Neurological profile: Spontaneous activity, reactivity, touch response, pain response and gait; Autonomic profile: Defecation and urination.

After a period of 24 and 72 h, lethality or death was observed.

Statistical analysis

All data were subjected to the analysis of variance and tested for significant differences by Duncan's multiple range tests (SAS Institute, Cary, NC). P values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Effect of PFP on body weights of mice

Figure 1 shows the body weights change of the mice during the experiment. The body weights of mice in the PFP supplemented groups (PFP-LG, PFP-MG and PFP-HG) were not significantly different from that in the control group (CG) before and after the experiment ($P > 0.05$), which means the PFP has no effect on body weight.

Effect of PFP on forced swimming capacity of mice

The forced swimming capacity test has been used extensively for the evaluation of the anti-fatigue properties of medicine (Ikeuchi et al., 2006; Jung et al., 2007; Tang et al., 2009). To standardize the workload and reduce the swimming time, weights at specific body weight percentages were added to the chest or tail of the animal. Figure 2 shows the effect of PFP on forced swimming capacity of mice. There are significant differences in the swimming time to exhaustion between the control group and each PFP supplemented group. The swimming time to exhaustion of the CG, PFP-LG, PFP-MG and PFP-HG were 352.6 ± 31.4 s, 487.3 ± 41.8 s, 546.9 ± 51.7 s, 617.1 ± 57.4 s, respectively. Thus, the swimming times to exhaustion of the PFP supplemented groups (PFP-LG, PFP-MG and PFP-HG) were significantly longer than that of the control group (CG) ($P < 0.05$). These results indicate that PFP could alleviate fatigue induced by forced swimming in mice and elevate the exercise tolerance.

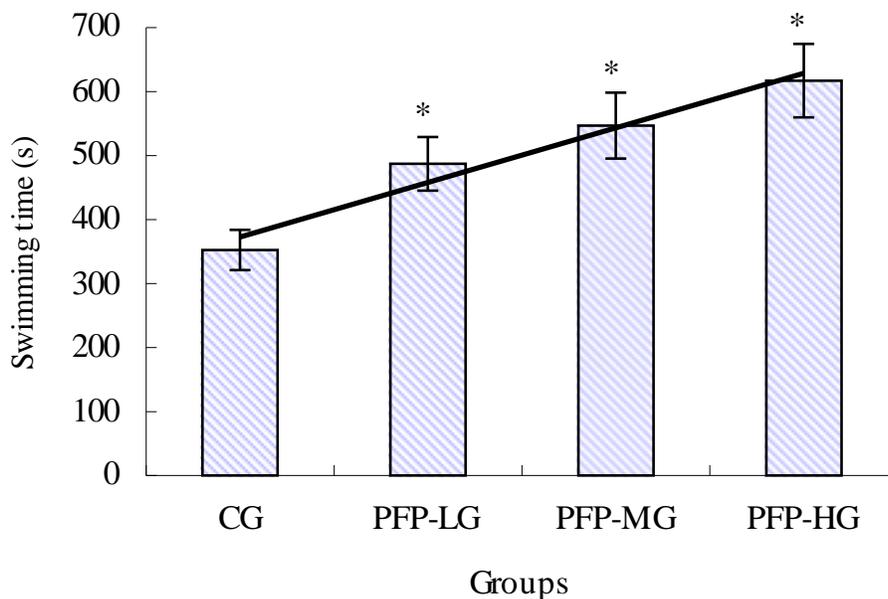


Figure 2. Effect of PFP on forced swimming capacity of mice ($P < 0.05$ as compared with the control group).

Effect of PFP on blood lactic acid of mice

Blood lactate 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. Therefore, the blood lactate acid is one of the important indicators for judging the degree of fatigue (Tadano et al., 2000; Wang et al., 2006). Many studies have indicated that if medicine could inhibit the accumulation of lactic acid and accelerate the clearance of lactic acid, it will have the anti-fatigue effects (Zhang et al., 2006; Tang et al., 2008; Ding et al., 2011). Figure 3 shows the effect of PFP blood lactic acid of mice. After swimming, BLA contents of the PFP supplemented groups (PFP-LG, PFP-MG and PFP-HG) were significantly lower than that of the control group (CG) ($P < 0.05$). These results indicate that PFP could effectively retard and lower the blood lactic produced, postpone the appearance of fatigue.

Effect of PFP on serum urea nitrogen of mice

Serum urea nitrogen (SUN) is another very sensitive index of fatigue status (Ma et al., 2008). Dynamophore in sports include sugar, fat and protein. When exercise time does not exceed 30 min protein seldom participates in energizing and SUN changes a little. Protein and amino acids have a stronger catabolic metabolism when body cannot obtain enough energy by sugar and fat catabolic metabolism, after a long time of exercise, SUN obviously increases at this time (Koo et al., 2004; Liu et al., 2005; Wang et al., 2008). Figure 4 shows the effect of PFP on

SUN of mice. After swimming, SUN contents of the PFP supplemented groups (PFP-LG, PFP-MG and PFP-HG) were significantly lower than that of the control group (CG) ($P < 0.05$). Reduced BUN contents with PFP treatment reflects reduced protein metabolism, which is indicative of enhanced endurance.

Effect of PFP on hepatic glycogen of mice

The liver converts lactate back to glycogen and releases glycogen into the blood. Energy for exercise is derived initially from the breakdown of glycogen, and later from circulation glycogen released by the liver and from non-esterified fatty acids. So increasing the hepatic glycogen storage conduces to enhancing the endurance capacity and locomotory capacity (Zhang et al., 2006; Tang et al., 2008; Zhao et al., 2009). Figure 5 shows the effect of PFP hepatic glycogen of mice. After swimming, hepatic glycogen contents of the PFP supplemented groups (PFP-LG, PFP-MG and PFP-HG) were significantly higher than that of the control group (CG) ($P < 0.05$). These results indicate that the anti-fatigue activity of PFP might be related to the improvement in the metabolic control of exercise and the activation of energy metabolism.

Acute toxicity study

Experiments were carried out on healthy Kunming mice. The behavior of the mice treated with PFP appeared normal. No toxic effect was seen even with the dose of 4 g/kg bodyweight and there were no lethality in any of the

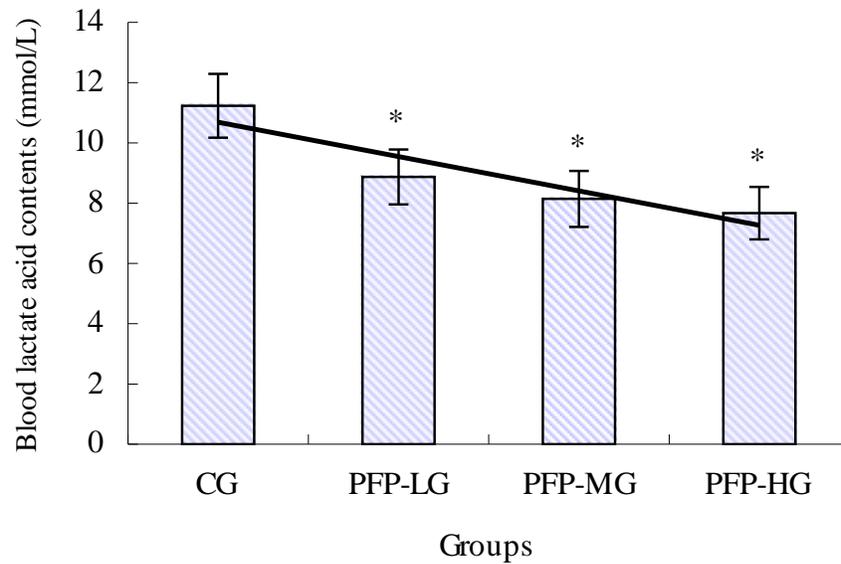


Figure 3. Effect of PFP on blood lactic acid of mice ($P < 0.05$ as compared with the control group).

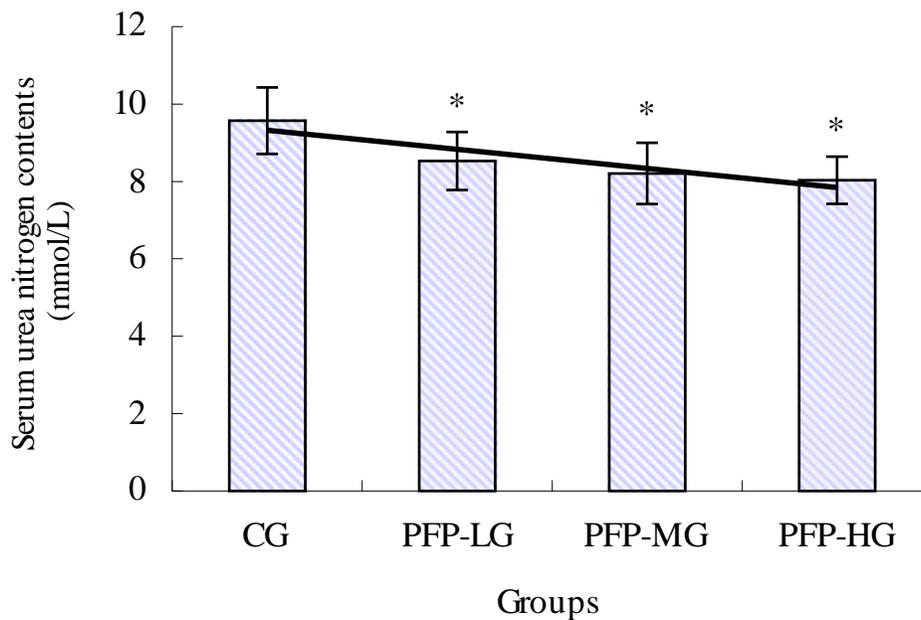


Figure 4. Effect of PFP on serum urea nitrogen of mice ($P < 0.05$ as compared with the control group).

group.

Conclusion

In conclusion, PFP supplementation could extend the swimming time to exhaustion of the mice, as well as increase the hepatic glycogen contents, while decreasing

the BLA and SUN contents, and these effects were dose-dependent. The strongest effect was seen with a 400 mg/kg dose. These indicated that PFP could alleviate fatigue induced by forced swimming in mice. However, further studies to clarify the detailed mechanisms involved in the anti-fatigue properties of the PFP are necessary.

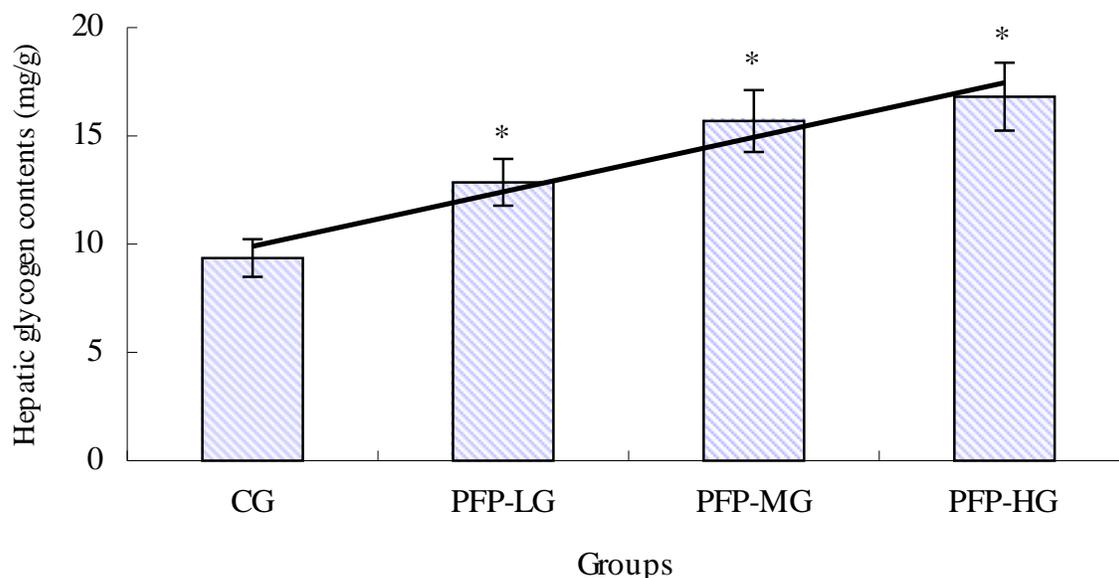


Figure 5. Effect of PFP on hepatic glycogen of mice ($P < 0.05$ as compared with the control group).

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