Anti-fatigue effects of polysaccharides extracted from *Portulaca oleracea* L. in mice

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*Portulaca oleracea* L. has been used as a food and medicinal plant for thousands of years in China. Polysaccharides extracted from *P. oleracea* L. (POP) are its main bioactive compound and have multiple pharmacological activities. However, anti-fatigue effects of POP have not yet been tested. This study was designed to investigate the anti-fatigue effects of POP in mice using the rotarod and forced swimming tests. The mice were randomly divided into four groups, namely normal control group, low-dose POP supplementation group, medium-dose POP supplementation group and high-dose POP supplementation group. The normal control group received distilled water and the supplementation groups received different doses of POP (75, 150 and 300 mg/kg, respectively). The POP or distilled water was administered orally and daily for 30 day. After 30 days, the rotarod and forced swimming tests were performed and then several biochemical parameters related to fatigue were determined. The data showed that POP prolonged the riding times and exhaustive swimming times of mice, decreasing blood lactic acid and serum urea nitrogen levels, as well as increasing the liver and muscle glycogen contents. These results indicated that POP had the anti-fatigue effects.

**Keywords:** Polysaccharides, *Portulaca oleracea* L., Fatigue, Riding times, Exhaustive swimming times, Blood lactic acid, Serum urea nitrogen, Glycogen

It is known that moderate exercise is useful for preventing illness and mental stress, but excessive exercise itself can result in stress and may cause fatigue or various types of damage to the body\(^1\). Several factors have been identified to induce fatigue during exercise. First, exercise promotes the consumption and depletion of energy sources, such as glycogen. Second, exercise causes the production and accumulation of metabolic products, such as lactic acid and ammonia in the body. Third, intense exercise can produce an imbalance between the body’s oxidation and anti-oxidation system, i.e., the accumulation of reactive free radicals will put the body in a state of oxidative stress and bring injury to the body by attacking large molecules and cell organs\(^2\text{-}^5\). Since there are very limited methods of treating fatigue in modern medicine, alternatives from natural substances and their respective mechanisms of action may be worth studying\(^6\). Previous studies have shown that the supplementation of natural substances can decrease the contribution of exercise-induced physical fatigue and improve the animal’s physiological capacities\(^7\text{-}^{12}\).

*Portulaca oleracea* L. is a common, herbaceous succulent annual weed, which is distributed extensively in temperate and tropical regions worldwide\(^13\). It has been used as a kind of food and medicinal plant for thousands of years in China. In traditional Chinese medicine, it has been used for treating dysentery with bloody stools, eczema, erysipelas and as febrifuge and antiseptic\(^14\). Pharmacological studies have shown that *P. oleracea* possess analgesic, anti-inflammatory\(^15\), antitumor\(^16\), antioxidant\(^17\), antiviral\(^14\), anti-hypoxic\(^18\), antidiabetic\(^19\), antitussive\(^20\) and neuropharmacological activity\(^21\). The plant contains various bioactive compounds, such as organic acids, alkaloids, coumarins, flavonoids, cardiac glycosides and polysaccharides etc\(^22\). Previous studies have shown that plant contains polysaccharides are main bioactive compounds, which have been reported to exhibit multiple pharmacological activities\(^23,24\). However, the effects of polysaccharides extracted from *P. oleracea* L. (POP) on physical fatigue have not been investigated thus far. Therefore, in this study, we have assessed the anti-fatigue effects of POP in mice.

**Materials and Methods**

**Plant material**

*Portulaca oleracea* L. plants were collected in fresh state at a local farm in Hangzhou city in July and identified by Dr Wang X in the Institute of Zhejiang Institute of Botany (Hangzhou, China). A voucher specimen (No. 140723) was deposited in the Herbarium of the Zhejiang Institute of Botany. Fresh plants were washed with distilled water and dried with air.
Chemicals and reagents
Commercial kits (special for animal testing) used for determination of blood lactic acid (BLA) and serum urea nitrogen (SUN) were purchased from Institute of Biological Engineering of Jianchen (Nanjing, China). Commercial kits (special for animal testing) used for determination of liver glycogen and muscle glycogen were purchased from Beijing Leadman Biochemistry Technology Co. Ltd. (Beijing, China). All other chemicals used were of analytical grade unless otherwise stated.

Preparation of polysaccharides extracted from *P. oleracea* L.
Polysaccharides extracted from *P. oleracea* L. (POP) were prepared as described previously\textsuperscript{18}. In brief, dried plants were ground to fine powder and 100 g of this powder was immersed in ten-fold distilled water (dH\textsubscript{2}O), boiled at 100°C for 9 h and then the water extract was collected. The process was repeated and the extracts were combined and concentrated on a vacuum rotary evaporator at 60°C. The concentrated solution was precipitated by addition of four-times its volume of volume 80% ethanol and the precipitate was washed in turn with 100% ethanol, 100% ether and acetone, respectively. After filtering and centrifuging, the precipitate was collected and vacuum-dried at 40°C, yielding the POP (9.41 g). The total carbohydrate was determined by the anthrone-sulfuric acid method and D-glucose was used as the standard.

Animals and treatments
Male Kunming mice, weighing 18 to 22 g, were purchased from Zhejiang Animal Husbandry Center (Hangzhou, China). Mice were housed in stainless-steel wire-bottom cages and maintained on a 12-h light/dark cycle (lights on 07:00-19:00 h) in a temperature- (22 ± 1°C) and humidity-controlled (55 ± 10% relative humidity) room, with free access to rodent pellet diet (Kehuang Feed Ltd, Hangzhou, China) and water. All experiments were conducted according to the guidelines of the Committee on the Care and Use of Laboratory Animals of the Zhejiang Police College (Hangzhou, China).

After 7 days of acclimation, 120 mice were randomly divided into four groups (30 mice per group). Group I or normal control (NC) group: the mice were allowed free access to a normal diet and were supplemented with distilled water; Group II or low-dose POP supplementation (LP) group: the mice were allowed free access to a normal diet and were supplemented with 75 mg/kg bw of POP; Group III or medium-dose POP supplementation (MP) group: the mice were allowed free access to a normal diet and were supplemented with 150 mg/kg bw of POP; and Group IV or high-dose POP supplementation (HP) group: the mice were allowed free access to a normal diet and were supplemented with 300 mg/kg bw of POP.

POP were dissolved in 2.0 mL of distilled water and the normal control received the same volume of distilled water. The rationale for the selection of the doses was based on our preliminary experiments. The POP or distilled water was administered orally and daily for 30 days. After 30 days, the rotarod and forced swimming tests were performed, followed by several biochemical parameters relating to fatigue.

Rotarod test
Ten mice were taken out from each group to make the rotarod test. Mice were first given 3 days to become acquainted with the rotarod instrument (Model YLS-4C, Jinan YiYan Technology Development Co., Ltd., Shandong, China) at the speed of 20 r/min for 60 s before the test. After the final treatment with POP or distilled water, the mice were placed in a rotarod instrument to induce fatigue at the speed of 20 r/min and the riding times (the time before the mice fell off the rod) were recorded.

Forced swimming test
Another ten mice were taken out from each group to make the forced swimming test. After the final treatment with POP or distilled water, the mice were placed individually in a swimming pool (50 × 40 × 50 cm) with 30 cm depth of water maintained at 25.0 ± 0.5ºC. The tail of each mouse was loaded with a bundle of lead pieces, which was 10% of its body weight. Exhaustion was determined by observing failure to swim, when the mice failed to rise to the surface of water to breathe within 10 s period\textsuperscript{25}. The exhaustive swimming times were recorded.

Determination of biochemical parameters relating to fatigue
The remaining ten mice were taken out from each group for biochemical parameters related to fatigue analyses. After the final treatment with POP or distilled water, the mice were forced to swim for 90 min without loads. After resting for an hour, mice were anesthetized with ethyl ether and sacrificed. The blood was collected in the test tube and quickly placed under ice cold condition. The whole blood samples were used for the determination of blood lactic acid (BLA). Serum was separated by centrifugation (2000 × g, 4°C, 10 min) for the determination of serum urea nitrogen (SUN). The
BLA content was determined based on the lactate dehydrogenase enzymatic method and the absorbance was read at 530 nm. SUN content was determined by the diacetyl monoxime colorimetric method and the absorbance was read at 520 nm.

The liver and gastrocnemius muscle tissues were quickly removed, washed with physiological saline and homogenized in ice-cold buffer. The homogenate was centrifuged for 15 min at 15000 × g at 4°C and the clear supernatant was used for the tissue glycogen contents measurements. Glycogen content was determined by the anthrone-sulfuric acid method and the absorbance was read at 620 nm.

Statistical analysis
Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Duncan’s multiple test. Results were expressed as mean ± SD. by group. Significant differences were determined at $P < 0.05$ by applying ANOVA, followed by SAS, version 6.12, Cary, NC.

Results and Discussion
Effects of POP on riding times and exhaustive swimming times
A direct measure of anti-fatigue effect is the increase in exercise tolerance. Rotarod test and forced swimming tests in the mice are widely used for the screening anti-fatigue agent and give a high reproducibility. The riding times and exhaustive swimming times can reflect the fatigue degree of movement and objectively the physical ability of body. As shown in Fig. 1, the riding times and exhaustive swimming times of mice in LP, MP and HP groups were significantly prolonged, compared to the NC group ($P < 0.05$). The riding times increased by 18.62%, 33.60% and 42.49%, respectively, while the exhaustive swimming times increased by 31.67%, 43.76% and 63.46%, respectively. These results indicated that POP had the anti-fatigue effects.

Effect of POP on blood lactic acid (BLA)
Lactic acid is produced by anaerobic glycolysis, which can be further degraded via the tricarboxylic acid cycle for the production of ATP by oxidative phosphorylation under normal circumstances. The accumulation of BLA during high-intensity exercise might change the internal pH value and cause the so-called acidosis, leading to the production of fatigue. Thus, BLA is one of the important indicators for judging the degree of fatigue. As shown in Fig. 2, the BLA levels of mice in LP, MP and HP groups significantly decreased, compared to the NC group ($P < 0.05$) and the blood lactic acid levels decreased by 29.87%, 46.15% and 74.07%, respectively. The results indicated that POP could effectively retard and lower the BLA produced, thus postpone the appearance of fatigue.

Effect of POP on serum urea nitrogen (SUN)
SUN is the metabolism outcome of protein and amino acid, which is another sensitive index of fatigue status. Protein and amino acids are metabolized to meet the energy requirement when the body cannot derive energy from carbohydrate and

![Fig. 1—Effect of POP on riding times and exhaustive swimming times of mice (NC, normal control; LP, low-dose POP supplementation; MP, medium-dose POP supplementation; HP, high-dose POP supplementation; * $P < 0.05$ as compared with the NC group)](image1)

![Fig. 2—Effect of POP on blood lactic acid (A), serum urea nitrogen (B) and liver and muscle glycogen (C) levels of mice (NC, normal control; LP, low-dose POP supplementation; MP, medium-dose POP supplementation; HP, high-dose POP supplementation; * $P < 0.05$ as compared with the NC group)](image2)
A positive correlation has been reported between urea nitrogen in vivo and exercise tolerance. As shown in Fig. 3, the SUN levels of mice in LP, MP and HP groups significantly decreased, compared to the NC group (P < 0.05) and the SUN levels decreased by 29.17%, 38.70% and 47.36%, respectively. Reduced SUN levels with POP treatment reflected reduced protein metabolism, which is indicative of enhanced endurance.

**Effect of POP on liver glycogen and muscle glycogen**

Energy for exercise is derived initially from the breakdown of glycogen and after strenuous exercise, glycogen reserves may exhaust, leading to insufficient energy supply or oxygen to the muscles, thus inducing muscle fatigue. So, glycogen is a sensitive index to test fatigue. As shown in Fig. 4, the content of liver and muscle glycogen of mice in LP, MP and HP groups significantly increased, compared to the NC group (P < 0.05) and the liver glycogen content increased by 27.88%, 39.56% and 57.94%, muscle glycogen contents increased by 24.41%, 38.97% and 44.61%, respectively. These results indicated that anti-fatigue activity of POP might be related to the improvement in the metabolic control of exercise and the activation of energy metabolism.

In conclusion, the results demonstrated that POP had the anti-fatigue effects, which prolonged the riding times and exhaustive swimming times of mice, decreasing BLA and SUN levels, as well as increasing the liver and muscle glycogen contents. However, further study is needed to elucidate the exact mechanism of the anti-fatigue activity of POP.

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**References**