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Original Research Article

Polysaccharides from Polygonatum odoratum strengthen antioxidant defense system and attenuate lipid peroxidation against exhaustive exercise-induced oxidative stress in mice

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Abstract

Purpose: *To investigate the potential effects of polysaccharides isolated from Polygonatum odoratum (PPO) on oxidative stress induced by forced swimming exercise in mice.*
Methods: *Mice were randomly divided into four groups: one control group and three PPO groups. The mice in the control group were administered with only distilled water, whereas the mice in the PPO groups were treated with different doses (150, 300, and 600 mg/kg) of PPO, respectively. The treatments were performed by oral gavage once daily for 28 days, and were followed by forced swimming test to determine exhaustive swimming. Furthermore, various biochemical parameters, including lactate dehydrogenase (LDH), creatine kinase (CK), superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and malondialdehyde (MDA) were assessed.*
Results: *PPO significantly ($p < 0.05$) prolonged exhaustive swimming time. It also significantly ($p < 0.05$) lowered LDH and CK levels in serum, as well as MDA levels in serum, liver and muscle, and also significantly ($p < 0.05$) improved SOD, GPx and CAT levels in serum, liver, and muscle.*
Conclusion: *PPO protects against oxidative stress induced by forced swimming exercise by strengthening antioxidant defense system and reducing lipid peroxidation. PPO may be suitable as a supplement for athletes and other physically active individuals for the improvement of exercise endurance and alleviation of fatigue.*

Keywords: *Polygonatum odoratum, Polysaccharides, Forced swimming test, Oxidative stress, Biochemical parameters*

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INTRODUCTION

Reactive oxygen species (ROS) play a significant role in maintaining homeostasis through cellular signals and immune function [1]. It is well known that exercise increases the utilization of oxygen in the body, and therefore enhances the production of ROS [2]. Specific sources of ROS during exercise include myeloperoxidase, xanthine oxidase, NADH oxidase, peroxisomal oxidative enzymes, and the mitochondrial electron transport chain (ETC) [3]. Under normal physiological conditions, ROS are neutralized through a complicated endogenous antioxidant defense system that is comprised of enzymatic and non-enzymatic antioxidants [4]. Antioxidant enzymes include glutathione peroxidase, catalase, and superoxide dismutase. The

principal non-enzymic antioxidants include reduced glutathione (GSH), vitamin C, and vitamin E [5]. An imbalance can be produced by exhaustive exercise in ROS and antioxidant defense, which results in oxidative stress [6]. This harmful status may lead to oxidative damage in all cellular components, including proteins, nucleic acids, lipids, and carbohydrates [7]. Exhaustive exercise-induced oxidative stress may result in muscle fatigue, muscle damage, and a reduction in physical performance [8]. Several studies have indicated that dietary antioxidants may prevent exercise-induced oxidative damage, as they are able to detoxify a number of peroxides directly through scavenging ROS that are generated during exercise or indirectly as modulators of intracellular pro- and anti-oxidant enzymes [9].

Polygonatum odoratum (Mill.) Druce belongs to the *Polygonatum* (Liliaceae) genus, and is widely grown in China's Northeast, Central and Southwest fields [10]. Its rhizome, also referred to as “YuZhu”, “WaiSheng”, “WeiRui”, or “LingDangCai” in Chinese, has been widely used as an ingredient or supplement in food supplies. Additionally, it is a well-known Chinese traditional medicine that functions in removing dryness, quenching thirst, promoting fluid secretion, and treatment of diverse diseases, including fatigue, hypo-immunity, diabetes, and cardiovascular disease [11,12]. Many studies have reported the biological activities of steroids, dipeptides, flavonoids, and polysaccharides isolated from *P. odoratum* [13]. Recent studies have demonstrated that polysaccharides are one of the principal bioactive components of *P. odoratum*, and the main monosaccharide compositions of polysaccharides are glucose, mannose, glucosamine, arabinose, rhamnose, and galactose [10]. Polysaccharides from *P. odoratum* (PPO) have been shown to possess antioxidant, antitumor, hypoglycemic, anti-fatigue, and immunomodulatory activities [10,14,15]. However, little is known about PPO's effects on oxidative stress induced by exhaustive exercise. Hence, the current study objective was to systematically investigate the potential effects of PPO on forced swimming exercise-induced oxidative stress in mice.

EXPERIMENTAL

Plant material

Dried rhizomes of *P. odoratum* were bought from a local herb store (Yancheng, China). The plant's authenticity was confirmed by Dr WY Shi, a plant taxonomist at School of Pharmaceutical Engineering, Yancheng Institute of Technology (Yancheng, China). A voucher specimen (YTU-PD-237) was kept in the herbarium of Yancheng Institute of Technology. The dried samples were crushed into a fine powder and then stored in an airtight container and maintained in a cool, dark, and dry place until further use.

Chemicals and reagents

The assay kit for lactate dehydrogenase (LDH) was bought from Beyotime Institute of Biotechnology (Shanghai, China), and that for creatine kinase (CK) was bought from Yuduo Biological Technology Co. (Shanghai, China). The assay kits for glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) were bought from Jiancheng Biotechnology Co. (Nanjing, China). Unless otherwise noted, all other chemicals and reagents used were of analytical grade and used without further purification. All solutions used were made with double distilled water.

Preparation of polysaccharides from *P. odoratum*

Polysaccharides from *P. odoratum* were isolated as previously reported [14,16] with small changes. Dried ground samples were extracted with distilled water at 90°C for approximately 2.5 h. The aqueous extract was filtered and centrifuged (2,000 × g for 10 min, at 20°C). The supernatant obtained was concentrated using a rotary evaporator at 60 °C under reduced pressure conditions, precipitated through adding ethanol to a final concentration of 80 % (v/v) and then incubated overnight. The resultant precipitate was dissolved in distilled water and deproteinized with the Sevag method, and again precipitated with ethanol to a final concentration of 80 % (v/v) and then incubated overnight. The precipitate was collected by centrifugation (2,000 × g for 10 min, at 20 °C), washed sequentially with ethanol, acetone, and ether, respectively, and then vacuum freeze-dried, resulting in crude polysaccharides.

Experimental animals

Male kunming mice, weighing 18 ± 2 g, were provided by the Laboratory Animal Breeding Center of Yancheng Institute of Technology and acclimatized to the test environment for one week before use. Mice were allowed to feed on the standard rodent pellet food and tap water *ad libitum*. The animals were housed in environmentally controlled conditions (humidity of 55 ± 5 %, temperature of 22 ± 1°C) with a 12:12 hour light:dark cycle. Animal procedures were

carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals [16], and approved by the Ethics Committee of Yancheng Institute of Technology (approval no. SYLL2015-0211).

Experimental design

After one week of adaptive feeding, the animals were divided into four groups at random (n = 8 for each group): control (C) group, low-dose PPO treated (PT150) group, medium-dose PPO treated (PT300) group and high-dose PPO treated (PT600) group. The mice in the PPO treatment groups were administered increasing doses of PPO (150, 300 and 600 mg/kg body weight) by oral gavage once daily for 28 days, and mice in the control group were administered distilled water instead of PPO at the equivalent volume (2.0 mL). After final treatment with PPO or distilled water, a forced swimming test was performed as described in the literature [17,18] with small changes. Briefly, the animals were permitted to rest for 30 min and were then loaded with a bundle of lead skin attached to their tails, which weighed ~5 % of their body weight. They were then placed in the swimming pool (50 × 50 × 40 cm) at a depth of 30 cm and kept at a temperature of 25 ± 1°C. The mice were determined as being exhausted by measuring the loss of coordinated movements and failure to come back to the surface of water within 10 seconds [17]. The exhaustive swimming times of the mice were used as a measure of exercise tolerance index.

Biochemical analysis

After the forced swimming test, the mice were sacrificed by decapitation under ether anesthesia, and the blood samples were collected in pre-sterilized tubes without the anticoagulant. Serum was obtained by the centrifugation (3,000 × g for 10 min) at room temperature, and stored at -20 °C until use. Following blood collection, tissue samples from hind-limb skeletal muscle and liver were excised rapidly and rinsed in ice-cold physiological saline, rapidly frozen in liquid nitrogen, and kept at -80 °C for further analysis.

All biochemical parameters, including LDH, CK, SOD, GPx, CAT, and MDA were determined using their respective manufacturers' analytical kits. Levels of LDH were measured using the enzymatic colorimetry method based on the fact that LDH can catalyze the reduction of pyruvate into lactic acid, while NADH oxidizes to NAD^+. Absorbance detection was set at 340 nm [19]. CK levels were determined using the molybdenum acid ammonium method based on the fact that CK catalyzes ATP and creatine to produce creatine phosphate (CP), which is quickly hydrolyzed to phosphoric acid. The addition of molybdenum acid ammonium produces phosphomolybdic acid, which can be further reduced to molybdenum blue. Enzymatic activity was calculated from the amount of inorganic phosphorus produced, and absorbance detection was set at 660 nm [20]. SOD levels were determined by the xanthine oxidase method, as the xanthine and xanthine oxidase reaction systems produce superoxide anion, which oxidize hydroxylamine to form nitrite. The ability of SOD to inhibit nitrite formation by superoxide anion was used as an indicator of enzyme activity. The absorbance detection was set at 550 nm [21]. GPx was measured by DTNB (5,5'-dithiobis-2-nitrobenzoic acid) method, as GPx can catalyze the reaction of H_2O_2 and reduced glutathione (GSH) to produce H_2O and oxidized glutathione (GSSG). Enzyme activity was calculated by measuring the GSH consumption in the enzymatic reaction. GSH reacts with DTNB to produce TNB (5-thio-2-nitrobenzoic acid) and GSSG, and with an absorbance detection at 412 nm, it can be calculated the amount of GSH [22]. CAT levels were determined by the ammonium molybdate method, as CAT decomposition of H_2O_2 can be interrupted by adding ammonium molybdate. The reaction of the remaining H_2O_2 with ammonium molybdate produced a yellow complex, and an absorbance detection set at 405 nm was used to calculate enzyme activity [23]. MDA contents were determined by the thiobarbituric acid method, as the reaction of the MDA with TBA produces a red MDA-TBA adduct, which has a maximum absorption peak of 535 nm [24]. The tissue protein contents were determined by the coomassie bright-blue stain method.

Statistical analysis

The data are presented as mean ± SD. Statistical analysis was carried out by one-way analysis of variance (ANOVA) using SPSS 18.0 software (SPSS Inc., Chicago, USA) and Dunnett's test. Significant differences was set at $p < 0.05$.

RESULTS

Effect of PPO on exhaustive swimming time

The exhaustive swimming time of the PT150, PT300, and PT600 groups were significantly longer than those of C group ($p < 0.05$, Figure 1).

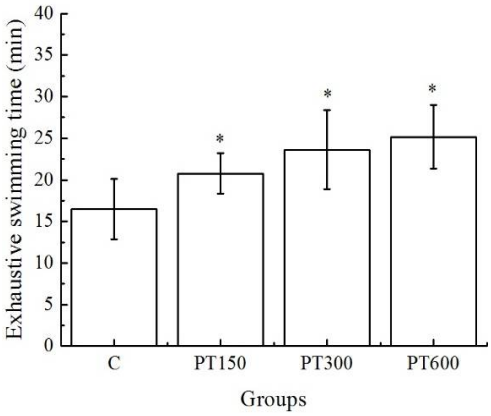

Figure 1: Effect of PPO on exhaustive swimming time of mice. Data are presented as mean ± SD (n = 8); *p < 0.05 as compared with group C

Effect of PPO on serum LDH and CK levels of mice

The LDH levels in the serum of the PT150, PT300, and PT600 groups, as well as the CK levels in serum of the PT300 and PT600 groups, were significantly lower than those of group C (p < 0.05) (Fig. 2).

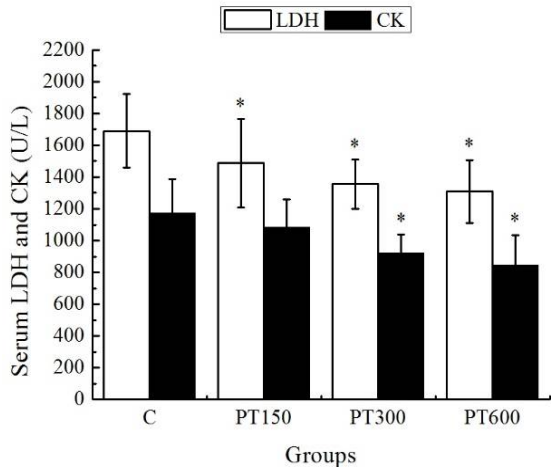

Figure 2: Effect of PPO on serum LDH and CK levels of mice. Data are presented as mean ± SD (n = 8); *p < 0.05 compared with group C

Effect of PPO on SOD, GPx and CAT levels in serum, liver, and muscle of mice

As can be seen from Figure 3a, SOD levels in the serum and liver of the PT150, PT300, and PT600 groups, as well as the SOD levels in muscle from the PT300 and PT600 groups, were significantly higher than those of the C group (p < 0.05). GPx levels in serum, liver, and muscle of the PT150, PT300, and PT600 groups were significantly higher than those of the C group (p < 0.05) (Figure 3b). The CAT levels in serum and muscle of the PT300 and PT600 groups, as well as the CAT levels in liver of the PT150, PT300, and PT600 groups, were significantly higher than those of group C (p < 0.05) (Figure 3c).

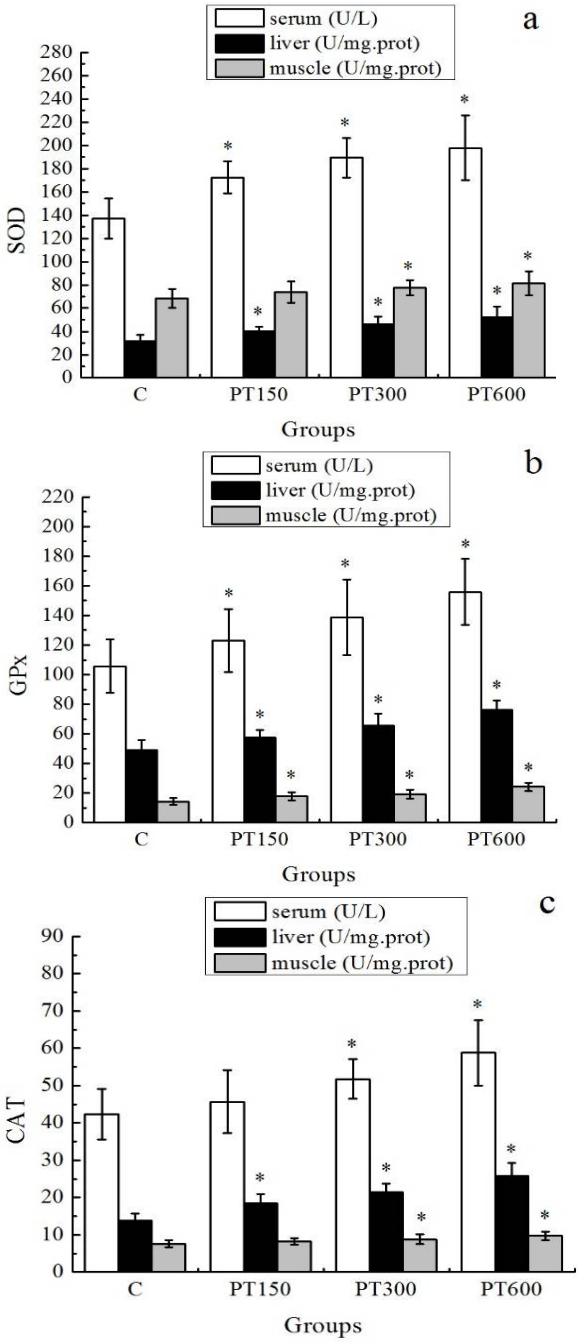

Figure 3: Effect of PPO on SOD, GPx and CAT levels in serum, liver, and muscle of mice. Data are presented as mean ± SD (n = 8); *p < 0.05 compared to group C

Effect of PPO on MDA content in serum, liver, and muscle of mice

In comparison with group C, the MDA content in the serum of the PT150, PT300, and PT600 groups, as well as the MDA content in the liver and muscle of the PT300 and PT600 groups, were significantly lower (p < 0.05) (Figure 4).

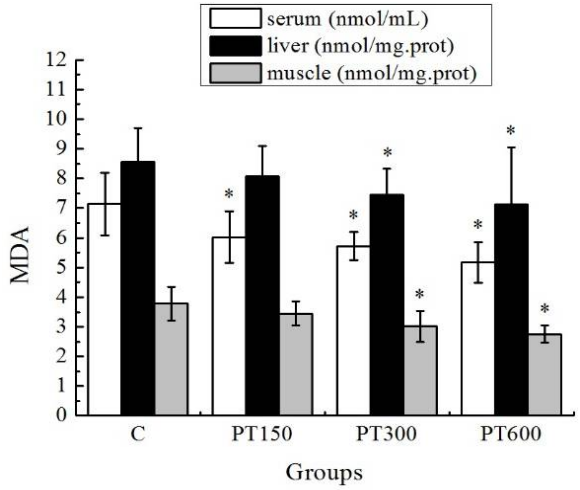

Figure 4: Effect of PPO on MDA content in serum, liver, and muscle of mice. Data are presented as mean ± SD (n = 8); *$p < 0.05$ compared with C group C

DISCUSSION

Forced swimming tests have been widely used to evaluate the anti-fatigue properties of natural products [25,26]. This method results in less mechanical stress and damage, and a better redistribution of blood flow in the tissues without significant changes in cardiac output and heart rate, which can in turn lead to an amount of damage that is caused by production of ROS [27]. In the present study, the significant increase in the exhaustive swimming time of various dose PPO treatment groups indicated that PPO has anti-fatigue properties.

It has been shown that exhaustive exercise causes a rise in oxygen consumption in tissues, leading to an increase in ROS production. Failure to get rid of ROS may result in a certain degree of muscle damage [28]. Serum LDH and CK are widely recognized as indicators of muscle damage after exhaustive exercise. As cytosolic enzymes, LDH and CK are physiologically abundant in organs' cells (e.g., heart, kidney, and skeletal muscles). However, during the process of exhaustive exercise, excessive ROS causes muscle cell damage, which increases LDH and CK in the blood [6]. The data from the present study showed that PPO significantly decreased serum LDH and CK levels of mice, implying that PPO had a preventive effect on exercise-induced muscle damage. This may be one of the reasons that PPO can extend the exhaustive swimming time of mice.

Numerous studies have demonstrated that antioxidant enzymes play an important function in preventing exercise-induced oxidative stress, and reduced activities or expressions of antioxidant enzymes may cause free radical damage in tissues [29]. As the first line of defense in the body against oxidative stress, SOD can completely inactivate superoxide anion radicals [30]. The two other key antioxidant enzymes in living body are GPx and CAT. GPx can convert GSH to GSSG, reducing toxic peroxide to non-toxic hydroxyl compounds, while promoting the decomposition of H_2O_2. GPx is regarded as an efficient protective enzyme on lipid peroxidation. CAT is mainly concentrated in the peroxisomes, which are responsible for the elimination of H_2O_2 produced from long-chain fatty acids metabolism [31]. In the present study, the significant increase in SOD, GPx, and CAT levels in serum, liver, and muscle of various dose PPO treatment groups indicated that PPO could strengthen the antioxidant defense system by upregulating the main antioxidant enzyme activities in response to the oxidative stress that is induced by the exhaustive exercise.

Excessive accumulation of ROS after exhaustive exercise can lead to lipid peroxidation of polyunsaturated fatty acids in biofilm and blood, and interfere with cell function [32]. Lipid peroxidation can cause fluidity loss and permeability increase of membranes, leading to the loss of cytosolic proteins [33]. Inhibition of lipid peroxidation is essential for maintaining normal cell function [34]. MDA is one of the end products of the unsaturated fatty acid peroxidation in phospholipids, and has been widely used as a marker of oxidative stress [4]. In the present study, PPO significantly decreased the MDA levels in serum, liver, and muscle of various dose PPO treatment groups, indicating that PPO can effectively attenuate lipid peroxidation.

CONCLUSION

The findings of the present study demonstrate that PPO has protective effects on exhaustive exercise-induced oxidative stress by strengthening the antioxidant defense system and reducing lipid peroxidation. Therefore, PPO has the potential to be developed as a supplement for athletes and other physically active individuals for improvement of exercise endurance and alleviation of fatigue.

DECLARATIONS

Acknowledgement

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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