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Full Length Research Paper

Effects of polysaccharides from *Pseudostellaria heterophylla* on exercise endurance capacity and oxidative stress in forced swimming rats

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The present study aimed to examine the effects of polysaccharides from *Pseudostellaria heterophylla* (PPH) on exercise endurance capacity and oxidative stress in forced swimming rats. Forty male Wistar rats were divided into four groups: a control group and three PPH treated groups. The rats of control group received 2 ml physiological saline solution; while the rats of PPH treated groups received the same volume of PFP of 100, 200 and 400 mg/kg body wt, respectively. Animals were administered orally and daily for 28 days, followed by being forced to undergo swimming endurance tests, with measurements taken of various biochemical parameters, including blood lactate, urea nitrogen (BUN), muscle glycogen, antioxidant enzymes (SOD and GPx) and malondialdehyde (MDA). Results showed that PPH administration significantly extended the swimming time of rats and displayed a lower level of blood lactate and BUN. Meanwhile, a higher level of muscle glycogen was displayed also. Furthermore, PPH could augment the level of antioxidant enzymes and effectively decrease the MDA content in the Muscle. Results of the study suggested that PPH could enhance exercise endurance and possessed protective effects against oxidative stress in rats undergoing strenuous exercise.

Key words: Polysaccharides from *Pseudostellaria heterophylla*, exercise endurance capacity, oxidative stress, rats.

INTRODUCTION

Pseudostellaria heterophylla (Radix Pseudostellariae) is an adaptogen in the Caryophyllaceae family, which is distributed widely in south China (Figure 1A) (Gong et al., 2001; Zhang et al., 2007). The dried roots of *P. heterophylla* named “Tongshen” or “Taizishen”, have been mostly used as one of the herbal ingredients in prescriptions of traditional Chinese medicines (TCM) to strengthen the body, and are commonly used to treat various lung and spleen diseases (Figure 1B) (Shen et al., 2008; Guo et al., 1993; Lin, 2004). As an estimate, more than 50 composite formulae of TCM contain *P. heterophylla* in China, such as Li Gan Zi Shen Tang (regulate the liver and enrich the kidneys decoction), etc. (Wu and Lin, 2004). Moreover, *P. heterophylla* has also been used as a tonic drug in China for the treatment of chronic fatigue syndrome (Sheng et al., 2009). Recently, studies on *P. heterophylla* including chemical ingredients and relevant pharmacological properties have been performed. Polysaccharides from *P. heterophylla* (PPH) have displayed obvious anti-infectious, anti-oxidative, and immunomodulating activities (Ng et al., 2004; Wong et al., 1994; Li and Fu, 2006; Cai et al., 2005). Unfortunately, the effects of PPH on physical exercise have never been investigated. Therefore, in the present study, we investigated the effects of PPH exercise endurance and exercise-induced oxidative stress in forced swimming rats.

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MATERIALS AND METHODS

Plant material

The dried roots of *P. heterophylla* (Radix Pseudostellariae) were purchased from JiuZhiTang herb shop, Luoyang, China (native to

(A) **(B)**

Figure 1. *Pseudostellaria heterophylla* (A) and its dried roots (Taizishen), (B) used in TCM application.

Shandong. cv. "YuoSheng No. 1").The authenticity of the plant was confirmed by Prof. Jiang MS, Luoyang Institute of Science and Technology, and a voucher specimen (HD179-6) was deposited in the Herbarium of Luoyang Institute of Science and Technology.

Preparation of polysaccharides from *Pseudostellaria heterophylla* (PPH)

The dried roots of *P. heterophylla* were grinded with electric mixer (JFSD-100, Shanghai Longtuo Co., Shanghai, China) before extraction. As described previously (Chen et al., 2007; Sheng et al., 2009), powdered *P. heterophylla* (400 g) were extracted three times by refluxing with 80% ethanol (1 L) at 90℃ for 2 to 3 h each time. After filtration, the gruffs were extracted again for three times with water (1.5 L) at 90℃ for 2 to 3 h each time. The extracted solution was condensed to 400 ml and deproteinated by applying the Sevag method. The solution was then added to absolute ethyl alcohol until the ethanol concentration was 80% and kept overnight, followed by filtration. The precipitate was dissolved with water (100 ml) and then absolute ethyl alcohol was added until the ethanol concentration was 80%, filtrated and repeated once again. The precipitate was washed with 95% ethanol, absolute ethyl alcohol and acetone by turns, and then dried at 50℃. Polysaccharides from *P. heterophylla* (PPH) were obtained.

Animals and experimental process

Male healthy Wistar rats weighing between 225 to 250 g were obtained from the Experimental Animal Center of Luoyang, China. A standard pellet diet and water were given *ad libitum*. Animals were maintained under a constant 12 h light and dark cycle and an environmental temperature of 21 to 23℃. All animal use procedures were in accordance with the Regulations of Experimental Animal Administration issued by State Committee of Science and Technology of the People's Republic of China on 14 November 1988.

Forty male Wistar rats were divided into four groups of ten animals each. All were administered orally and daily for 28 days. Group 1 received 2 ml physiological saline solution as control group; Groups 2, 3 and 4 received 100, 200 and 400 mg/kg body wt. of PPH as treated groups, respectively. This dose was chosen based on our previous studies. Since there is no reference compound or positive control available, Group 1 received only physiological saline solution, which was also the solution used to dissolve the PPH, and was assigned as control.

Exercise endurance capacity

The swimming endurance capacity was assessed 10 h after the last treatment. The details of this apparatus were reported by Lee et al. (2009) as the acrylic plastic pool (90 × 60 ×60 cm) filled with 40 cm deep of water maintained at 28 ± 1℃. Rats were forced to swim in the water, and the endurance was defined as the time they kept swimming actively until the animal submerged in water without movement. To diminish stress, all rats had been accustomed to swimming with repeated short-term swimming sessions for a week before experiment. This exercise in familiarization of swimming prior to treatments allowed minimizing the scattering of data and improving the accuracy of data on swimming capacity.

Blood and tissue sample preparation

Immediately after swimming exercise, the rats were anaesthetized with pentobarbital sodium (5 mg/100 g body wt, i.p.). Blood was obtained from the orbital sinus for lactate and urea nitrogen (BUN) content measurements. Hind-limb skeletal muscle was quickly excised and homogenized immediately with homogenizer. fitted with teflon plunger, in ice-chilled 10% KCl solution (10 ml/g of tissue). The suspension was centrifuged at 671×g at 4℃ for 10 min and clear supernatant was used for glycogen, malondialdehyde

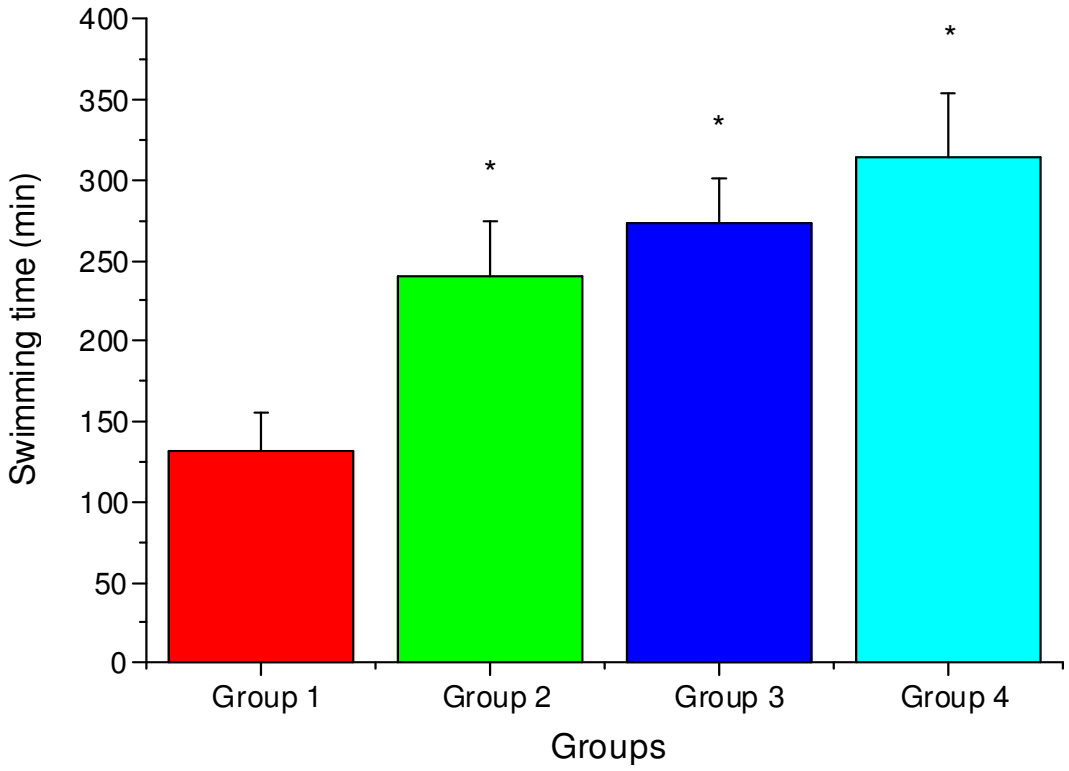

Figure 2. Effects of PPH on swimming time of rats. Data are presented as mean ± SD of ten rats per group.*P < 0.05, compared with the control group (Group 1).

Table 1. Effects of PPH on lactate and urea nitrogen (BUN) in the blood of rats.

Groups	Number	Lactate (mmol/L)	BUN (mmol/L)
Group 1	10	4.29±0.23	7.89±0.86
Group 2	10	3.85±0.31*	6.64±0.91*
Group 3	10	3.20±0.17*	6.23±0.74*
Group 4	10	2.93±0.26*	6.12±0.46*

Data are presented as mean ± SD of ten rats per group.*P < 0.05, compared with the control group (Group 1).

(MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPx) content measurements.

Lactate was determined according to the procedures provided by the kits (Beijing Leadman Biochemistry Technology Co. Ltd., Beijing, China). SUN was determined according to the procedures provided by the kits (Shanghai Chengzheng Biomedical Engineering Co. Ltd., Shanghai, China). Glycogen, malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GPx) were determined according to the procedures provided by the kits (Institute of Biological Engineering of Nanjing Jianchen, Nanjing, China).

Statistical analysis

All analyses were performed using SPSS V11.5 software for Windows. All the data were given as means ± standard deviations (SD) of three replications. Data were analyzed by one-way ANOVA. Whenever ANOVA was significant, further comparisons between groups were evaluated by using the Dunnett's t-test. The level of statistical significance adopted was P < 0.05.

RESULTS

Endurance swimming time

As shown in Figure 2, swimming times in all the PPH treated groups (Groups 2, 3 and 4) were significantly longer compared with that of the control group (Group 1) (P < 0.05).

Lactate and urea nitrogen (BUN) contents

As shown in Table 1, lactate and urea nitrogen (BUN) contents in all the PPH treated groups (Groups 2, 3 and

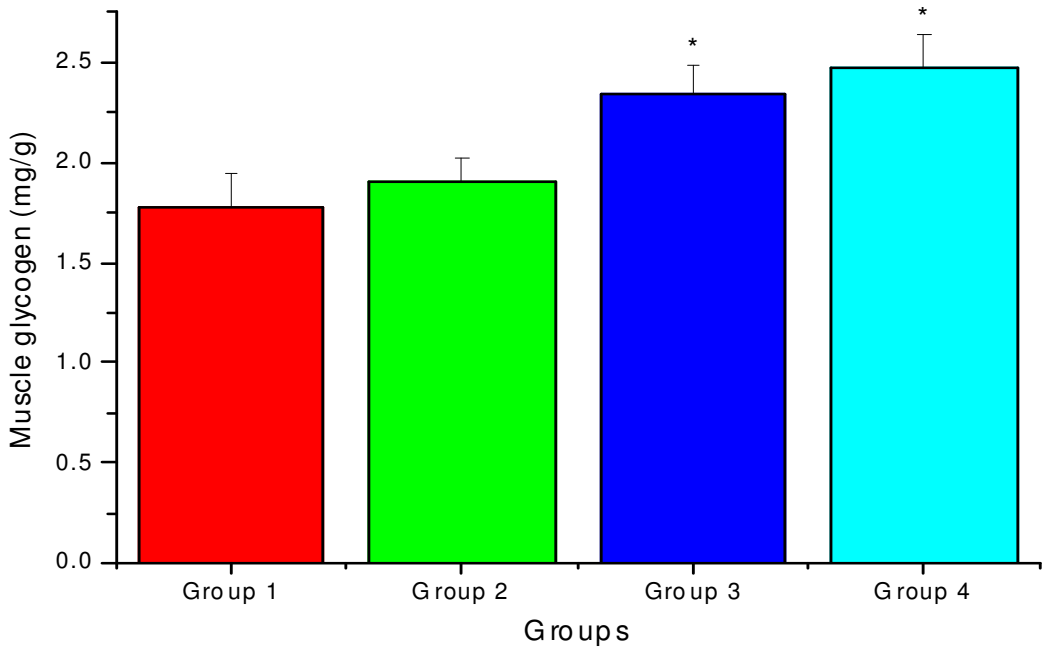

Figure 3. Effects of PPH on muscle glycogen in the skeletal muscle of rats. Data are presented as mean ± SD of ten rats per group.*P < 0.05, compared with the control group (Group 1)

4) were significantly lower compared with that of the control group (group 1) (P < 0.05).

Muscle glycogen content

As shown in Figure 3, muscle glycogen content in the PPH treated groups (Groups 3 and 4) were significantly higher compared with that of the control group (Group 1) (P < 0.05).However, there were no differences in muscle glycogen content between the Groups 2 and 1 (P >0.05).

MDA content

As shown in Figure 4, MDA content in all the PPH treated groups (Group 2, 3 and 4) were significantly lower compared with that of the control group (Group 1) (P < 0.05).

Antioxidant enzymes contents

As shown in Table 2, antioxidant enzymes (SOD and GPx) contents in all the PPH treated groups (Group 2, 3 and 4) were significantly higher compared with that of the control group (group 1) (P < 0.05).

DISCUSSION

The dried roots of *P. heterophylla* (Taizishen) are amongst the most popular health-promoting herbs in China, with their use dated back more than 2000 years, and was recorded in Shen Nong's Materia Medica written in the Han dynasty (Liu, 2000) Polysaccharides have long been recognized as the main active component of *P. heterophylla* (Liu and Wang, 1993). In this study, we examined the effects of polysaccharides from *P. heterophylla* (PPH) on exercise endurance capacity and oxidative stress in forced swimming rats.

Forced swimming time and spontaneous running wheel activity are usually adopted to represent the physical work capacity and fatigue condition of animals (Lee et al., 2009; Feng et al., 2009). In the current study, the results showed that PPH administration significantly extended the swimming time of rats (Figure 2). The findings demonstrated that PPH could improve exercise endurance. The swimming exercise is known to induce blood biochemical changes, including blood lactate and BUN (Chen et al., 2002). Lactate serves as an energy source in highly oxidative tissues. Many organs such as liver and heart, and tissues such as skeletal muscle, help to remove lactate from the blood, but intense exercise can increase lactate production (Wei et al., 2010). SUN is a sensitive index to evaluate the bearing capability when human bodies suffer from a physical load and caused by catabolism of proteins and amino acids (Wu, 1999). Protein and amino acids have a stronger catabolic metabolism when body cannot obtain enough energy by sugar and fat catabolic metabolism. Therefore, there is a positive correlation between the urea nitrogen *in vivo* and the exercise tolerance. In the current study, the results showed that PPH administration significantly decreased blood lactate and BUN contents of rats after swimming

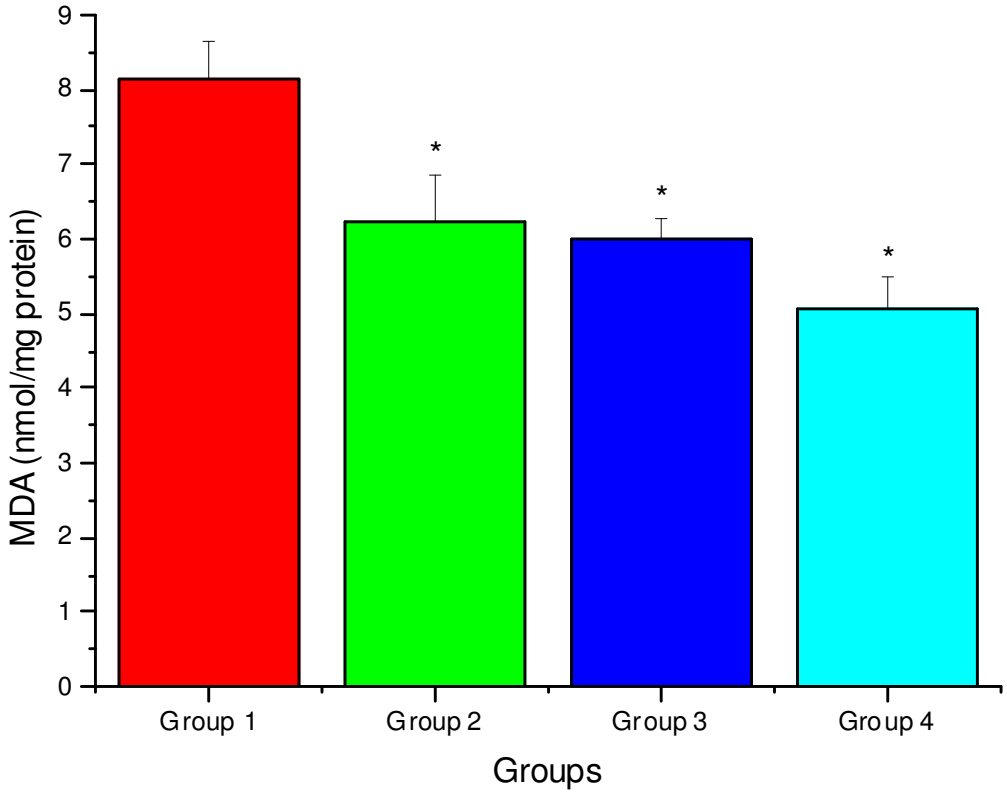

Figure 4. Effects of PPH on MDA in the skeletal muscle of rats. Data are presented as mean ± SD of ten rats per group. *P < 0.05, compared with the control group (Group 1).

Table 2. Effects of PPH on antioxidant enzymes (SOD and GPx) in the skeletal muscle of rats.

Groups	Number	SOD (U/mg protein)	GPx (U/mg protein)
Group 1	10	101.31±11.42	6.57±1.38
Group 2	10	148.53±10.78*	9.86±1.85*
Group 3	10	162.87±13.46*	12.39±1.16*
Group 4	10	169.52±12.24*	16.34±1.72*

Data are presented as mean ± SD of ten rats per group.*P < 0.05, compared with the control group (Group 1).

exercise (Table 1).The findings demonstrated that PPH could effectively attenuate the increase of blood lactate and reduce catabolic decomposition of protein for energy. Energy for exercise is derived initially from the break-down of glycogen, and after strenuous exercise glycogen reserves will exhaust, which can cause insufficient energy supply or oxygen to the muscles thus inducing muscle fatigue (Shan et al., 2010). In the current study, the results showed that PPH administration significantly increased muscle contents of rats after swimming exercise (Figure 3). The findings demonstrated that PPH might improve glycogen reserves and reduced the glycogen consume during exercise.

Growing evidence indicates that exercise increases oxygen utilization and causes formation of free radicals and reactive oxygen species (ROS) (Wetzstein et al., 1998; Minato et al., 2003). Moreover, strenuous aerobic exercise is associated with oxidative stress (Ji, 1995; Kerksick and Willoughby, 2005).Oxidative stress may progress to oxidative damage involving cellular proteins (contractile, structural, and enzymatic), lipids, DNA, and other molecules in ways that might lead to abnormal cellular function (Tharakan et al., 2005; Lu et al., 2006). MDA is secondary product generated during the oxidation of polyunsaturated fatty acids (Misra et al., 2009). In the current study, the results showed that PPH administration significantly decreased MDA contents of rats after swimming exercise (Figure 4), The findings demonstrated that PPH could effectively reduce lipid peroxidation. Free radicals are produced by lipid peroxidation derived from oxygen, and the first line of defense against them is SOD and GPx. The increase in SOD and GPx in muscle would

indicate an up-regulation of the defense mechanism to try to cope with an enhanced production of superoxide anion radicals. This in turn might help to down-regulate the production of lipid peroxides or oxidative stress (Reid, 2008; Lee et al., 2009). In the current study, the results showed that PPH administration significantly increased SOD and GPx contents of rats after swimming exercise (Table 2), The findings demonstrated that PPH was able to up-regulate antioxidant enzymes to protect against oxidative stress-induced injury during exercise..

Conclusion

The present study clearly showed that polysaccharides from *P. heterophylla* (PPH) administration significantly extended the swimming time of rats and displayed a lower level of blood lactate and BUN. Meanwhile, a higher level of muscle glycogen was displayed also. Furthermore, PPH could augment the level of antioxidant enzymes and effectively decrease the MDA content in the muscle, which suggested that PPH could enhance exercise endurance and possessed protective effects against oxidative stress in rats undergoing strenuous exercise.

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