African Journal of Agricultural Research Vol. 7(17), pp. 2632-2638, 5 May, 2012
Available online at http://www.academicjournals.org/AJAR
DOI: 10.5897/AJAR11.1206
ISSN 1991-637X ©2012 Academic Journals

Full Length Research Paper

Effects of *Gynostemma pentaphyllum* (Thunb.) Makino polysaccharides supplementation on exercise tolerance and oxidative stress induced by exhaustive exercise in rats

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Accepted 30 March, 2012

The purpose of this study was to evaluate the effects of *Gynostemma pentaphyllum* (Thunb.) Makino polysaccharides (GPMP) supplementation on exercise tolerance and oxidative stress induced by exhaustive exercise. Male rats were divided into 5 groups of 10 animals each. The first, second, third and forth groups designated as PGP treatment group was administered with GPMP of 50, 100, 200 and 400 mg/kg body weight by gavage every day, respectively. The fifth group designated as control group was administered with the equal volume of distilled water. After 30 days, exhaustive swimming exercise of rats was performed, and then the exhaustive swimming time, liver glycogen level, antioxidant enzymes activities and MDA concentrations were determined. Results of the aforementioned study showed that GPMP supplementation prolonged exhaustive swimming time and improved liver glycogen reserve, which suggested that GPMP supplementation improved exercise tolerance. Furthermore, GPMP supplementation could promote increases in the activities of super oxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPH-Px), and reduce MDA concentrations, which suggested that PGP supplementation reduced oxidative stress induced by exhaustive exercise.

Key words: *Gynostemma pentaphyllum* (Thunb.) Makino, polysaccharides, exercise tolerance, oxidative stress, rat.

INTRODUCTION

Gynostemma pentaphyllum (Thunb.) Makin (botanical name) or Jiao-gu-lan (Chinese name), a perennial creeping herb distributed in Japan, Korea, China, and Southeast Asia, is praised in China as Xian-cao (the herb of immortality) (Yin et al., 2004). For hundreds of years, this plant has been regarded as a traditional Chinese medicine or a folk medicine used for heat clearing, detoxification, and as an anti-tussive and expectorant for relieving cough and chronic bronchitis (Zhang and Sun, 1994; Megalli et al., 2005; Liu et al., 2008). In recent decades, pharmacological studies have revealed that *G. pentaphyllum* (Thunb.) Makin has many bioactivities, including antimicrobia, anti-cancer, anti-aging, anti-fatigue, anti-ulcer, hypolipidemic and immuno-modulatory qualities (Wang et al., 2002; Rujjanawate et al., 2004; Megalli et al., 2006; Yeo et al., 2008; Srichana et al., 2011; Schild et al., 2010; Long, 2010). *G. pentaphyllum* (Thunb.) Makin contains saponins, polysaccharides, flavonoids, organic acids and trace elements and other chemicals (Zhang et al., 2007). To date, its biological activities are mainly attributed to saponins (triterpene glycosides or gypenosides) (Kao et al., 2008; Xie et al., 2010). However, recent studies have suggested that the polysaccharide from *G. pentaphyllum* (Thunb.) Makin

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(GPMP) also exhibit significant bioactivities, including anti-aging, anti-fatigue and improving immune competence (Luo and Wang, 2005; Chi et al., 2008; Yang et al., 2008). In addition, GPMP showed scavenging activity against superoxide radicals and inhibitory effects on selfoxidation of 1,2,2-phentriol (Wang and Luo, 2007a), suggesting its potential as an antioxidant.

Exhaustive exercise is often associated with an increase in the production of free radicals and reactive oxygen species (ROS) in various tissues, which results in oxidative stress (Sen et al., 1994; Aguilóet al., 2005; Morillas-Ruiz et al., 2006). Oxidative stress can induce adverse effects on health and well being. Even moderate exercise may increase ROS production exceeding the capacity of antioxidant defences (Aguiló et al., 2005). Over the last 30 years, it has been indicated that ROS can lead to the destruction of tissue and cell macromolecules such as lipids, proteins and nucleic acids (Perse et al., 2009; Miyazaki et al., 2001). The mechanisms implicated in ROS formation during exhaustive exercise are thought to include catecholamine autoxidation, the reperfusion of ischemic tissues, prostanoid metabolism, altered calcium homeostasis, and mechanical stress (particularly in the case of eccentric exercise, which has been shown to initiate cytokine activity, thus, playing a causal role in inflammation (Jówko et al., 2011). Growing evidence has indicated that exogenous antioxidants, primarily obtained as nutrients or nutritional supplements, may help to counteract the exhaustive exercise-induced oxidative stress (Peake and Suzuki, 2004; Watson et al., 2005; Gomez-Cabrera et al., 2006).

GPMP has been reported to be a potential antioxidant (Wang and Luo, 2007a; Shi et al., 2009). However, the effects of GPMP supplementation on oxidative stress induced by exhaustive exercise are still poorly understood. Therefore, the purpose of this study was to investigate the effects of GPMP supplementation on exercise tolerance and oxidative stress induced by exhaustive exercise.

MATERIALS AND METHODS

Plant materials and reagents

The commercial diagnostic kits of liver glycogen, super oxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPH-Px) and malondialdehyde (MDA) were purchased from the Jianchen Bioengineering Institute (Nanjing, China). Other chemicals and biochemicals were of analytical grade and were purchased from Sigma Chem. Co. (St. Louis, MO, USA) and Changsha Pharmaceutical Co. (Changsha, China) unless otherwise indicated. Dried *G. pentaphyllum* (Thunb.) Makin was purchased from the Huangshan Pharmaceutical Company (Huangshan, China) and authenticated by Dr. Fang J. Y. A voucher specimen (No. 037009) was deposited in the herbarium of the Laboratory of Pharmaceutical Sciences, Huangshan University (Huangshan, China). The materials were ground separately into powder using a miller before extraction of the crude polysaccharides.

Gynostemma pentaphyllum (Thunb.) Makin polysaccharides preparation

The *G. pentaphyllum* (Thunb.) Makin powder (250 g) was extracted with 95% ethanol at 50°C for 6 h, dried, and then extracted with distilled water at 95°C for 1.5 h twice. After each extraction, the soluble polymers were separated from residues by Wltration, and extracts were combined, concentrated and dialyzed against running water for 48 h. The aforementioned extract was submitted to graded precipitation with four volumes of ethanol and the mixture was kept overnight at 4°C to precipitate the polysaccharides. The precipitate was collected by centrifugation, washed successively with ethanol and ether, and dried at reduced pressure (Wang and Luo, 2007b). Then, the crude polysaccharides from *G. pentaphyllum* (Thunb.) Makin (GPMP) was obtained. The content of polysaccharide was determined by the phenol-sulphuric acid method (Dubois et al., 1956) and expressed as glucose equivalents. The glucose equivalent was 217.4 µg/mg of GPMP.

Animals and grouping

Male rats each weighing 180 to 220 g of Sprague Dawley strain were obtained from the Experimental Animal Center of Anhui Province, China (SPF grade) and acclimated for 1 week. They were housed in a standard animal facility under controlled environmental conditions at room temperature of 22 ± 2°C and 12-h light-dark cycle, and received a standard pellet diet and water *ad libitum*. All animal (used in this experiment) handling procedures were performed in strict accordance with the P. R. China legislation for the use and care of laboratory animals, with the guidelines established by Institute for Experimental Animals of Huangshan University, and were approved by the College committee for animal experiments. The rats were divided into 5 groups of 10 animals each. The first, second, third and forth group designated as PGP treatment group was administered with GPMP of 50, 100, 200 and 400 mg/kg body weight by gavage daily for 30 consecutive days, respectively. GPMP in the present study were dissolved in a small amount of distilled water. The fifth group designated as control group was administered with the equal volume of distilled water by gavage daily for 30 consecutive days. Body weights were measured by electronic balance at 0 (pre-trial) and 30 days after the administration of GPMP.

Exhaustive swimming exercise

Exhaustive swimming exercise was carried out as described in the literature (Yildiz et al., 2009; Perse et al., 2009). Thirty consecutive days later, the rats exercised in acrylic plastic pool (90 × 45 × 45 cm) filled with water (28 ± 1°C) to a depth of 37 cm. The rats were loaded with a steel washer weighing approximately 7% of their body weight attached to the tails, which forced the rats to maintain continuous rapid leg movement. The uncoordinated movements and staying under the water for 10 s without swimming at the surface were accepted as the exhaustion criteria of the rats (Dawson and Horvath, 1970). Exhaustive swimming time was recorded as minute for each rat.

Tissue preparation

All animals were sacrificed by decapitation while under ketaset anaesthesia (20 mg/kg body weight) immediately at the end of swimming exercise on the 30th day. The gastrocnemius muscle and liver tissues were collected. The liver tissue was immediately rinsed with ice-cold 0.9% NaCl solution, dried with paper towels and weighed for determining the liver index. Then, all the tissues were

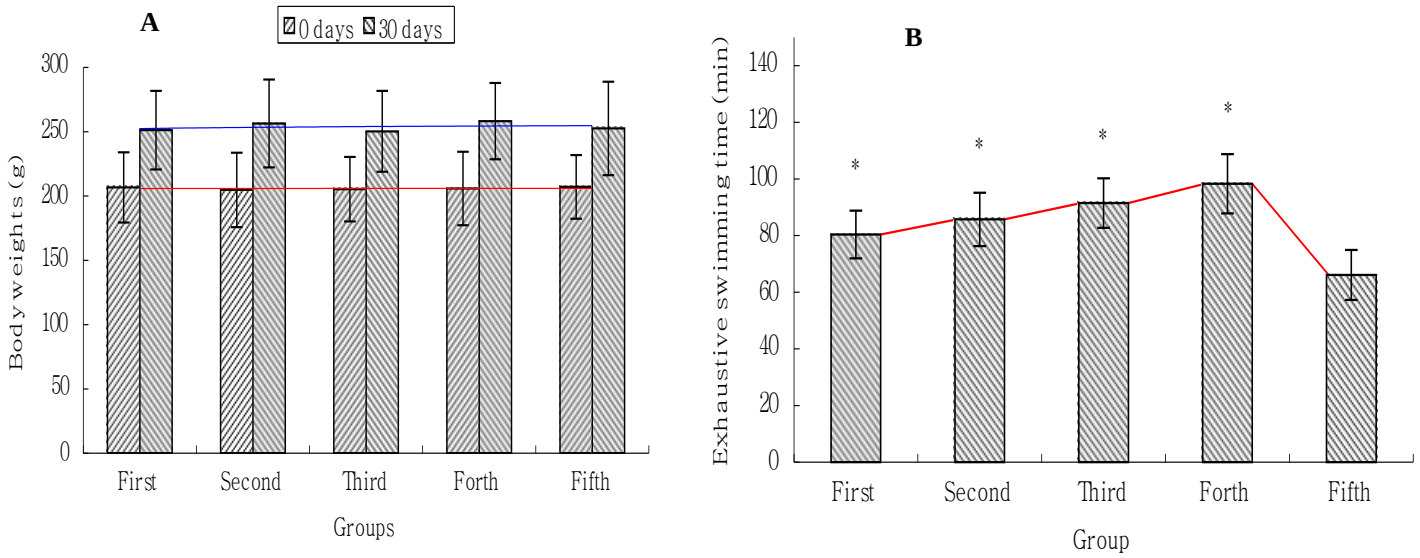

Figure 1. Effects of GPMP on the body weights and exhaustive swimming time of the rats. Body weights (A) and exhaustive swimming time (B). Note, values are expressed as means ±SD of ten; *P < 0.05, compared with the fifth (control) group.

refrigerated at -20°C and within 2 h of refrigeration, the tissues were processed for determining the liver glycogen level, antioxidant enzymes activities and MDA concentrations in muscle tissue.

Analytical method

The liver glycogen level, SOD, GSH-Px, CAT activities and MDA concentrations were determined using commercial diagnostic kits following the manufacturer's instructions. The liver index as calculated according to the following formula:

$$\text{Liver index (LI)} = \frac{\text{Liver weight (g)}}{\text{Body weight (g)}} \times 100\%$$

Statistical analysis

All values were presented as the means ± SD. Statistical comparisons of the differences were performed using one way analysis of variance for repeated measures combined with the Newman-Keuls post hoc test. P values below 0.05 were considered statistically significant.

RESULTS

Effects of GPMP on the body weights and exhaustive swimming time of the rats

As shown in Figure 1, the body weights in all GPMP treatment groups were of no significant difference compared with the fifth (control) group (P > 0.05) at 0 (pre-trial) and 30 days after the treatment of GPMP, which meant GPMP had no effect on body weights. After 30 days of treatment with GPMP, the exhaustive swimming time was much longer in all GPMP treatment groups compared with the fifth (control) group (P < 0.05), and the increase ratios were 21.54% (first group), 29.64% (second group), 38.32% (third group) and 48.69% (forth group), respectively.

Effects of GPMP on the liver index and liver glycogen levels of the rats

As shown in Figure 2, after 30 days of treatment with GPMP, the liver index in the experimental groups were of no significant difference compared with the fifth (control) group (P > 0.05), so GPMP had no significant effect on the liver index. The liver glycogen levels were much higher in all GPMP treatment groups compared with the fifth (control) group (P < 0.05), and the increase ratios were 27.63% (first group), 39.47% (second group), 47.37% (third group) and 56.58% (forth group), respectively.

Effects of GPMP on the antioxidant enzymes activities in muscle tissue of the rats

As shown in Figure 3, the SOD activities of the second, third and forth groups were significantly higher than that of the fifth (control) group (15.92, 23.46 and 36.14% greater, respectively) (P < 0.05), while the first group had no significant differences (P > 0.05), compared with the fifth (control) group. The GSH-Px activities were much higher in all GPMP treatment groups compared with the

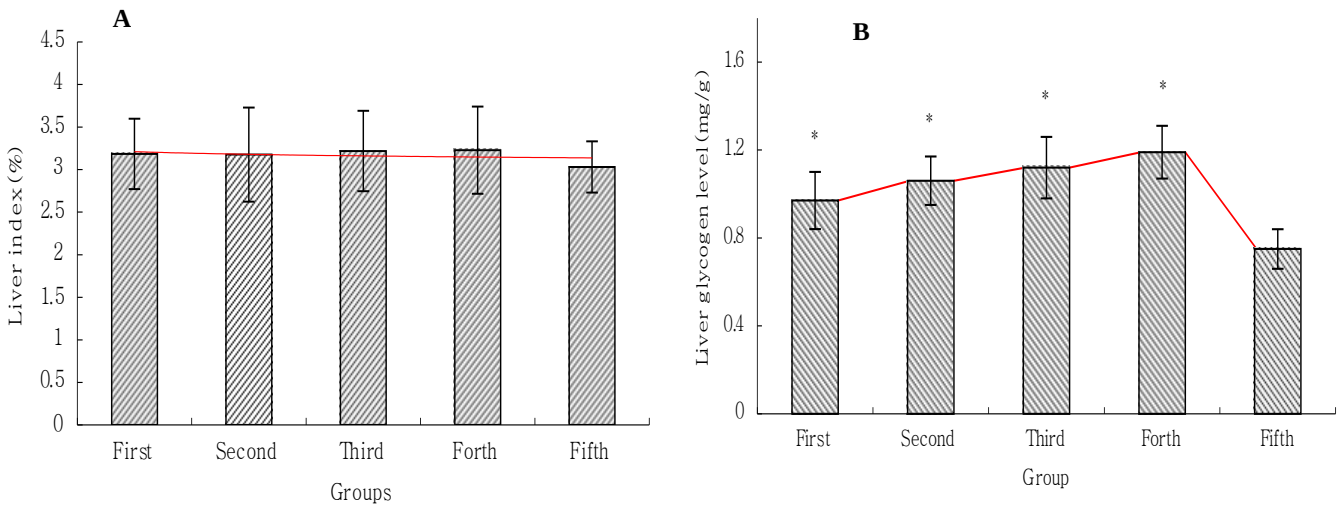

Figure 2. Effects of GPMP on the liver index and liver glycogen levels of the rats. liver index (A) and liver glycogen (B). Note, values are expressed as means ±SD of ten; *P < 0.05, compared with the fifth (control) group.

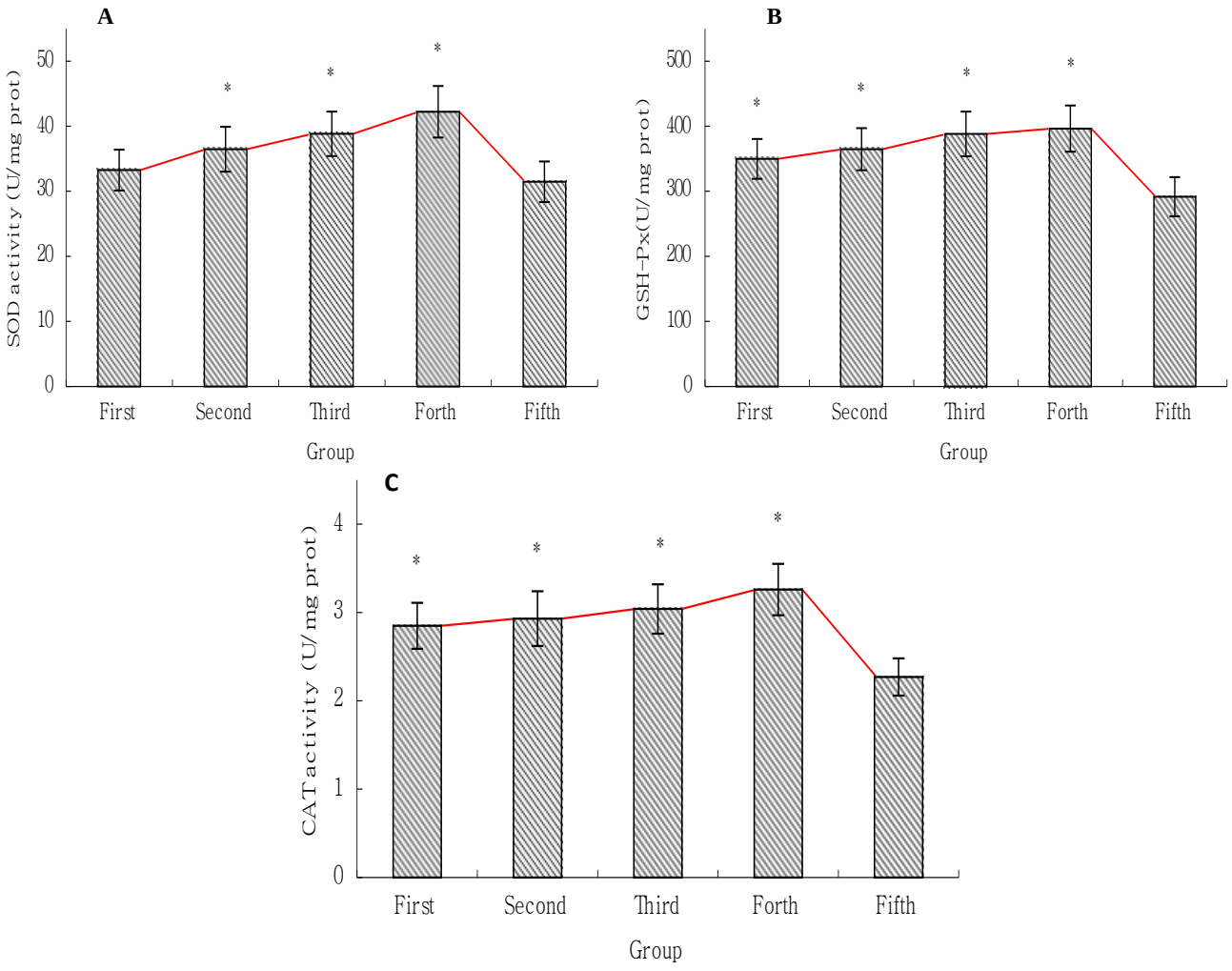

Figure 3. Effects of GPMP on the antioxidant enzymes activities in muscle tissue of the rats. SOD (A), GSH-Px (B) and CAT (C). Note, values are expressed as means ±SD of ten; *P < 0.05, compared with the fifth (control) group.

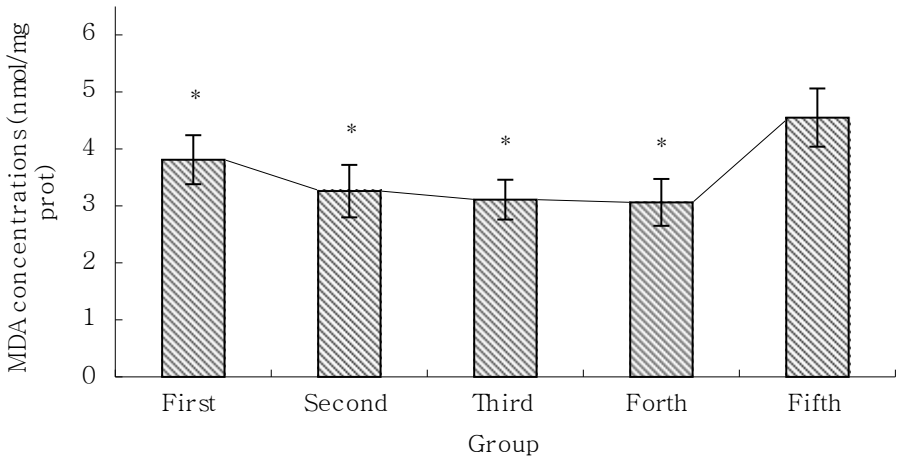

Figure 4. Effects of GPMP on the MDA concentrations in muscle tissue of the rats. Note, values are expressed as means ±SD of ten; *P < 0.05, compared with the fifth (control) group.

control group (P < 0.05), and the increase ratios were 19.97% (first group), 25.01% (second group), 33.09% (third group) and 35.95% (forth group), respectively. The CAT activities were much higher in all GPMP treatment groups compared with the control group (P < 0.05), and the increase ratios were 44.91% (first group), 39.20% (second group), 39.20% (third group) and 41.82% (forth group), respectively.

Effects of GPMP on the MDA concentrations in muscle tissue of the rats

As shown in Figure 4, the MDA concentrations were much lower in all PGP treatment groups compared with the control group (P < 0.05), and the decrease ratios were 19.42% (first group), 39.57% (second group), 46.30% (third group) and 48.69% (forth group), respectively.

DISCUSSION

The current study determined the effects of *G. pentaphyllum* (Thunb.) Makino polysaccharides (GPMP) supplementation on exercise tolerance and oxidative stress induced by exhaustive exercise. This premise is based on the fact that recent studies have demonstrated the antioxidant effects of PGP. Swimming exercise was chosen as a suitable model since it is a natural behaviour of rodents. The method causes less mechanical stress and injury, and leads to a better redistribution of blood flow among tissues without significant variations in cardiac output and heart rate which in turn may minimize the magnitude of injury caused due to the generation of ROS (Aydin et al., 2007). The present study demonstrated that the GPMP supplementation prolonged exhaustive swimming time, which suggested that GPMP supplementation influenced the performance of exhaustive exercise and improved exercise tolerance. Furthermore, GPMP supplementation improved liver glycogen reserve. It was known that endurance capacity of body was markedly decreased if the energy was exhausted. As glycogen was the important resource of energy during exercise, the increase of glycogen stored in liver is an advantage to enhance the endurance of the exercise (Ding et al., 2009). In this study, the prolongation of the exhaustive swimming time exhibited by the rats administered with GPMP may be related to the improvement in the physiological function or the activation of energy metabolism. Exhaustive exercise is associated with accelerated generation of reactive oxygen species (ROS) that results in oxidative stress. To combat the deleterious effects of ROS, the body has some complex internal protective mechanisms like enzymatic defenses, which include primary antioxidative enzymes like super oxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPH-Px) and non-enzymatic defenses like vitamin C, vitamin E, ubiquinol co-enzyme Q-10 and reduced glutathione (Gupta et al., 2009). SOD dismutates superoxide radicals to form H_2O_2 and O_2. GPH-Px is an enzyme responsible for reducing H_2O_2 or organic hydroperoxides to water and alcohol, respectively.

CAT catalyses the breakdown of H_2O_2 to form water and O_2 (Shan et al., 2011). It is known that antioxidant enzymes exhibit synergistic interactions by protecting each other from specific free radical attacks (Perse et al., 2009). The significant decrease in the activities of SOD, GPH-Px and CAT in the muscle tissue after forced swimming may be an indication of exercise-induced oxidative threat (Misra et al., 2009). Malondialdehyde

(MDA) has been the most widely used parameter for evaluating oxidative damage to lipids, although, it is known that oxidative damage to amino acids, proteins and DNA also causes release of MDA. Previous studies had indicated that exhaustive exercise causes an increase in MDA and the MDA increasing due to excess oxygen radical reacting polyunsaturated acid in the muscle (Misra et al., 2009; Sun et al., 2010). The present study demonstrated that the GPMP supplementation can promote increases in the activities of these antioxidant enzymes (SOD, GPH-Px and CAT) and reduce lipid per-oxidation. These observations suggested that GPMP supplementation had beneficial effects on attenuating the oxidative stress induced by exhaustive exercise.

Conclusion

The present study clearly showed that GPMP supplementation influenced the performance of exhaustive exercise and improved exercise tolerance. Moreover, GPMP supplementation could promote increases in the activities of SOD, GPH-Px and CAT, and reduce lipid per-oxidation, which suggested that GPMP supplementation was beneficial in enhancing the antioxidant status and inhibiting oxidative stress induced by exhaustive exercise.

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