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Protective Effects of Crude Polysaccharide from *Gynostemma pentaphyllum* on Swimming Exercise-Induced Oxidative Stress in Rat

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Abstract: The present study examined the effects of crude Polysaccharide from *Gynostemma pentaphyllum* (PGP) against oxidative stress induced by swimming exercise. Male rats were administered PGP (50, 100, 200 and 400 mg kg^{-1}) by gavage every day. After 30 days, swimming exercise of rat was performed in acrylic plastic pool. The results showed that PGP treatment prolonged exhaustive swimming time and improved liver glycogen reserve which suggested that PGP treatment influenced the performance of exhaustive exercise and improved exercise tolerance. Moreover, PGP treatment can promote increases in the activities of Super Oxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPH-Px) and reduce lipid per-oxidation which suggest that PGP treatment was beneficial in enhancing the antioxidant status and inhibiting oxidative stress induced by exhaustive exercise.

Key words: Crude polysaccharide from *Gynostemma pentaphyllum*, oxidative stress, swimming exercise, rat, SOD, China

INTRODUCTION

Gynostemma pentaphyllum (THUNB.) MAKINO also called Jiaogulan, praised in China as xiancao, the herb of immortality, a perennial creeping herb distributed in Japan, Korea, China and Southeast Asia (Yin *et al*., 2004). The plant was first described in 1406 CE by Zhu Xiao who presented a description and sketch in the book Materia Medica for Famine as a survival food rather than a medicinal herb (Zhang and Sun, 1994). The earliest record of Jiaogulan's use as a drug comes from herbalist Li Shi-Zhen's book Compendium of Meteria Medica published in 1578 (Liu *et al*., 2008; Mishra and Joshi, 2011). In traditional Chinese medicine it is indicated for heat clearing, detoxification and as an anti-tussive and expectorant for relieving cough and chronic bronchitis (Megalli *et al*., 2005). In recent decades, pharmacological studies have revealed that among many bioactivities of *G. pentaphyllum*, these include 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* 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* (PGP) 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, PGP showed scavenging activity against superoxide radicals and inhibitory effects on selfoxidation of 1, 2, 2-phentriol (Wang and Luo, 2007), suggesting its potential as an antioxidant.

Exhaustive exercise is associated with accelerated generation of Reactive Oxygen Species (ROS) that results in oxidative stress (Sen *et al*., 1994; Aguilo *et al*., 2005; Morillas-Ruiz *et al*., 2006) which can induce adverse effects on health and well being. Even moderate exercise may increase ROS production exceeding the capacity of antioxidant defences (Aguilo *et al*., 2005). The ROS has been shown to induce damage in all cellular macromolecules such as lipids, proteins and DNA (Miyazaki *et al*., 2001). Growing evidence has indicated that exogenous antioxidants, primarily obtained as

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nutrients or nutritional supplements may help to counteract the exercise-induced oxidative stress (Peake and Suzuki, 2004; Watson *et al*., 2005; Gomez-Cabrera *et al*., 2006; Misra *et al*., 2006). PGP has been reported to be an potential antioxidant (Wang and Luo, 2007; Shi *et al*., 2009). However, the effect of PGP supplementation on oxidative stress induced by exhaustive exercise is still poorly understood. Therefore, the purpose of this study was to investigate the effects of crude polysaccharide from *G. pentaphyllum* against oxidative stress induced by swimming exercise in rats.

MATERIALS AND METHODS

Plant materials: Dried *G. pentaphyllum* was purchased from the Huangshan Pharmaceutical Company (Huangshan, China) and authenticated by Dr. Fang JY. A voucher specimen (No.: 037009) was deposited in the herbarium of the Laboratory of Pharmaceutical Sciences, Huangshan University (Huangshan, China). The dried *G. pentaphyllum* were ground separately into powder using a miller before extraction of the crude polysaccharides.

Experimental animals: Male rats each weighing 180-220 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 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 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.

Extraction of crude polysaccharides from *G. pentaphyllum*: The *G. pentaphyllum* 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 filtration and extracts were combined, concentrated and dialyzed against running water for 48 h. The above 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, 2007). Then, the crude Polysaccharides from *G. pentaphyllum* (PGP) were 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^{-1} of PGP.

Experimental design: The rats were divided into 5 groups of 10 animals each. The 1st, 2nd, 3rd and 4th group designated as PGP treatment group was administered with PGP of 50, 100, 200 and 400 mg kg^{-1} body weight by gavage every day for 30 consecutive days, respectively. PGP in the present study were dissolved in a small amount of distilled water. The 5th group designated as control group was administered with the equal volume of distilled water by gavage every day for 30 consecutive days.

Swimming exercise program: Swimming exercise was carried out as described in the literature (Yildiz *et al*., 2009). About 30 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 tail which forced the rat to maintain continuous rapid leg movement. The uncoordinated movements and staying under the water for 10 sec 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^{-1} body weight) immediately at the end of swimming exercise on the 30th day gastrocnemius muscle and liver were collected. All the tissues were refrigerated at -20°C and within 2 h of refrigeration, the tissues were processed to determine the liver glycogen level, antioxidant enzymes activities and MDA concentrations.

Analytical method: The liver glycogen level, SOD, GSH-Px, CAT activities and MDA concentrations were determined using commercial diagnostic kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) following the manufacturer's instructions.

Statistical analysis: All values are 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. The $p<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

As shown in Fig. 1, the exhaustive swimming time was much longer in all PGP treatment groups compared to the 5th (control) group ($p<0.05$), the increase ratios were 21.54% (1st group), 29.64% (2nd group), 38.32% (3rd group) and 48.69% (4th group), respectively.

As shown in Fig. 2, the liver glycogen levels were much higher in all PGP treatment groups compared to the 5th (control) group ($p<0.05$), the increase ratios were 27.63% (1st group), 39.47% (2nd group), 47.37% (3rd group) and 56.58% (4th group), respectively.

As shown in Fig. 3, the SOD activities of the 2nd-4th 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 1st group had no significant differences ($p>0.05$), compared to the 5th (control) group.

As shown in Fig. 4, the GSH-Px activities were much higher in all PGP treatment groups compared to the control group ($p<0.05$), the increase ratios were 19.97% (1st group), 25.01% (2nd group), 33.09% (3rd group) and 35.95% (4th group), respectively.

As shown in Fig. 5, CAT activities were much higher in all PGP treatment groups compared to the control group ($p<0.05$), the increase ratios were 44.91% (1st group), 39.20% (2nd group), 39.20% (3rd group) and 41.82% (4th group), respectively. As shown in Fig. 6, the MDA concentrations were much lower in all PGP treatment

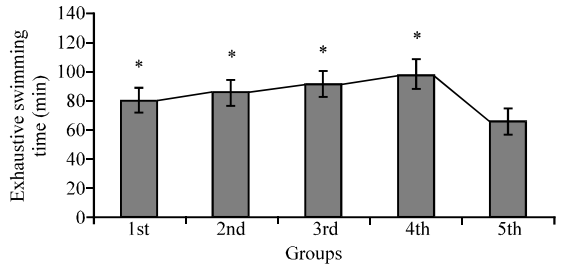

Fig. 1: Effects of PGP on the exhaustive swimming time of the rats. Values are expressed as means±SD of ten; *p<0.05, compared with 5th (control) group

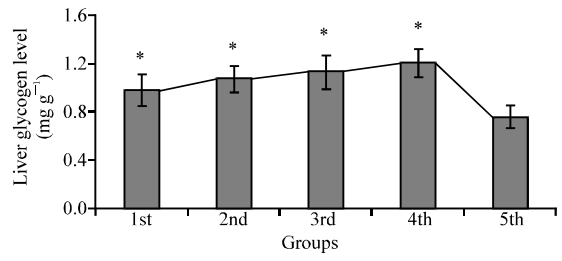

Fig. 2: Effects of PGP on the liver glycogen levels of the rats. Values are expressed as means±SD of ten; *p<0.05, compared with 5th (control) group

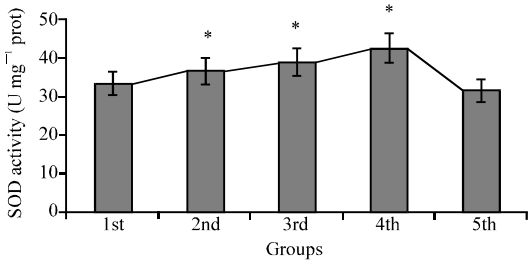

Fig. 3: Effects of PGP on the SOD activities of the rats. Values are expressed as means±SD of ten; *p<0.05, compared with 5th (control) group

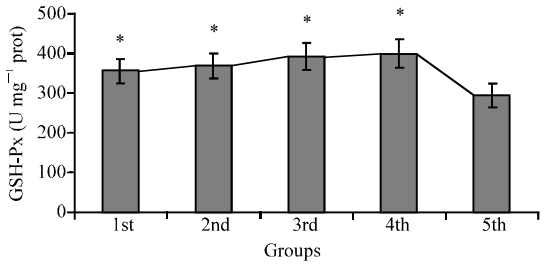

Fig. 4: Effects of PGP on the GSH-Px activities of the rats. Values are expressed as means±SD of ten; *p<0.05, compared with 5th (control) group

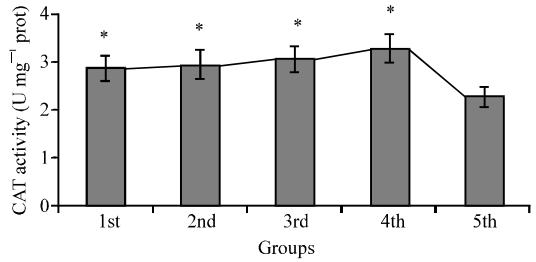

Fig. 5: Effects of PGP on the CAT activities of the rats. Values are expressed as means±SD of ten; *p<0.05, compared with 5th (control) group

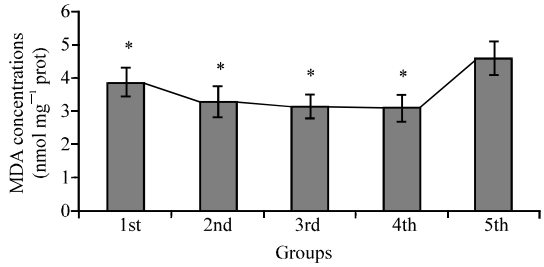

Fig. 6: Effects of PGP on the MDA concentrations of the rats. Values are expressed as means±SD of ten; *p<0.05, compared with 5th (control) group

groups compared to the control group ($p<0.05$), the decrease ratios were 19.42% (1st group), 39.57% (2nd group), 46.30% (3rd group) and 48.69% (4th group), respectively.

The current study determined the effect of crude Polysaccharide from *Gynostemma pentaphyllum* (PGP) on swimming exercise-induced oxidative stress. This premise is based on the fact that recent studies have demonstrated the antioxidant effects of PGP. Swimming 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 PGP treatment prolonged exhaustive swimming time which suggested that PGP treatment influenced the performance of exhaustive exercise and improved exercise tolerance. Furthermore, PGP treatment 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 increasing of glycogen stored in liver is 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 PGP 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 and 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). 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 PGP treatment can promote increases in the activities of these antioxidant enzymes (SOD, GPH-Px and CAT) and reduce lipid per-oxidation. These observations suggest that PGP treatment had beneficial effects on attenuating the oxidative stress induced by exhaustive exercise.

CONCLUSION

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

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