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 Jiagulan, 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 Jiagulan’s use as a drug comes from herbalist Li Shi-Zhen’s book Compendium of Materia 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, hypolipidemia and immuno-modulatory qualities (Wang et al., 2002; Rujjana et al., 2004; Megalli et al., 2006; Yeo et al., 2008; Srivastava et al., 2011; Schilh 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 glycosides) (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 self-oxidation of 1, 2, 2-phenentrol (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; Agullo 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 (Agullo 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 animals (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 21.74 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 groups (p<0.05), compared with the 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$_2$O$_2$ and O$_2$. GPH-Px is an enzyme responsible
for reducing H$_2$O$_2$ or organic hydroperoxides to water
and alcohol, respectively. CAT catalyses the breakdown of
H$_2$O$_2$ 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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