

EFFECTS OF POLYSACCHARIDES FROM *GYNOSTEMMA PENTAPHYLLUM* (THUNB.), MAKINO ON PHYSICAL FATIGUE

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*E-mail: zhangmenglw@163.com**Abstract**

Background: *Gynostemma pentaphyllum* (Thunb.) Makino has been reported to have a wide range of health benefits in Chinese herbal medicines. Polysaccharides from *Gynostemma pentaphyllum* (PGP), has been identified as one of the active ingredients responsible for its biological activities. Although many pharmacological activities of PGP have received a great deal of attention, there is limited evidence for the anti-fatigue effects of PGP. The purpose of this study was to investigate the effects of polysaccharides from PGP on physical fatigue.

Materials and method: The rats were divided into four groups, with 10 animals per group: control (C), group, low-treated (LT), group, medium-treated (MT), group, and high-treated (HT), group. The C group received distilled water, while LT, MT and HT groups were given various doses of PGP (100, 200, 400 mg/kg·d). After 30 days, forced swimming test was carried out in an acrylic plastic pool, then the exhaustive swimming time of rats and some biochemical parameters related to fatigue were measured. The data obtained showed that PGP could extend the exhaustive swimming time of the rats, as well as decrease the blood lactic acid (BLA), and blood urea nitrogen (BUN), concentrations, and increase the hemoglobin, liver glycogen and muscle glycogen concentrations.

Result: The data obtained showed that different doses of PGP could extend the exhaustive swimming time of the rats, as well as decrease the BLA and BUN concentrations, and increase the hemoglobin, liver glycogen and muscle glycogen concentrations, which suggests that PGP had significant anti-fatigue effects on rats.

Conclusion: PGP may be of use as a potential anti-fatigue agent, but there is a need for further research on long-term use in order to show its positive effects on physical fatigue.

Key words: polysaccharides from *Gynostemma pentaphyllum* (Thunb.) Makino; physical fatigue; forced swimming test; rats

Introduction

Fatigue (also called exhaustion, tiredness, lethargy, languidness, languor, lassitude, and listlessness) can be both physical and mental (Newsholme et al., 1992; Fu et al., 2010). Physical fatigue is generally defined as a reduction in capacity to perform physical work as a function of preceding physical effort. Mental fatigue is generally defined as the deterioration of mental performance due to preceding exercise of mental or physical activity (Berrios, 1990; Newsholme and Blomstrand, 1996). Since modern medicine has limited therapies for fatigue and those offered by it have strong side-effects, potential alternatives from traditional medicine and their respective mechanisms of action are worth investigating (Tharakan et al., 2005).

Gynostemma pentaphyllum [Thunb], Makino (GP), (Jiaogulan-Chinese name), is a kind of perennial herbs climbing shrub of *G. pentaphyllum* genus of gourd family, and it mainly grows in the southern provinces of China, Japan, Korea and Southeast Asia (Hu et al., 2009; Liao et al., 2011). Ancient Chinese medical texts celebrated GP for their wide range of health benefits including strengthening life force of the body. For hundreds of years, this herb has been commonly used in Chinese herbal medicines with the effects of clearing heat, detoxification, and as an anti-tussive and expectorant for relieving cough and chronic bronchitis (Zhang et al., 2005). A large number of pharmacological studies have demonstrated that GP possesses antimicrobial, anti-cancer, anti-aging, anti-fatigue, anti-ulcer, hypoglycemic, hypolipidemic and immunomodulatory qualities (Yeo et al., 2008; Yuan et al., 2010; Srichana et al., 2011; Im et al., 2012). GP has been shown to contain many biologically active phytochemicals like saponins, polysaccharides and flavonoids.

To date, its biological activities are mainly attributed to saponins. However, recent studies have reported that the polysaccharides from GP (PGP), also exhibit significant bioactivities. Qian et al. (1999), report that PGP could markedly enhance the clearance of charcoal particles, increase the natural killer (NK) cells activity in liver cancer mouse model. Wang and Luo (2007), report that crude PGP exhibited scavenging capacities against hydroxyl radical and superoxide radicals. Chen et al. (2011), demonstrated that the sulfated polysaccharide from GP have distinct antitumor activities. Chi et al. (2012), found that PGP could increase antioxidant enzyme activities some of antioxidant enzyme activities, and decrease MDA level in muscle of exercised mice. Li et al. (2012), also found that 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 of mice. Niu et al. (2013) also report that a novel heteropolysaccharide from GP (GPP-TL) exhibited scavenging capacities against hydroxyl, peroxy, and DPPH radicals. However, there is limited evidence for the anti-fatigue effects of PGP. Therefore, the purpose of this study was to investigate the effects of PGP supplementation on physical fatigue using a forced swimming test in rats.

Materials and methods**Plant materials and reagents**

Dried *Gynostemma pentaphyllum* (Thunb.) (voucher number TH36721) Makino was purchased from a local shop (Tangshan, China), and authenticated by Dr. Fang JY, Department of Biological Engineering, Tangshan Teacher's College (Tangshan, China). D-glucose (AR class), was purchased from Shanghai Shensuo Reagents Co. (Shanghai, China). Blood lactic acid (BLA) and hemoglobin assay kits were purchased from Jianchen Chemical Co., Ltd. (Nanjing, China). Blood urea nitrogen (BUN), liver glycogen and muscle glycogen assay kits were purchased from Kehua Bio-engineering Co., Ltd. (Shanghai, China). The other chemicals and reagents were analytical grade and purchased from Tangshan Pharmaceutical Co. (Tangshan, China).

Preparation of polysaccharides from *Gynostemma pentaphyllum* (Thunb.), Makino

Polysaccharides from *Gynostemma pentaphyllum* (Thunb.), Makino (PGP) were prepared using methods previously described with some modifications (Wang and Luo, 2007; Wang et al., 2012). Briefly, the dried GP were ground up carefully and turned into powder. The resulting powder (250 g), was extracted with 95% ethanol at 50°C for 6 hrs, dried, and then extracted with distilled water at 95°C for 1.5 hrs 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 hrs. 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 precipitates collected by centrifugation (2000×g for 10 min, at 20°C), were washed by ethanol and ether for two times and dried under reduced pressure. The polysaccharide concentration was determined by the phenol-sulphuric acid method using d-glucose as a standard.

Experimental animals and care

Male Spargue-Dawley rats, weighing 180-220 g, were purchased from the Experimental Animal Center of Hebei Province (Shijiazhuang, China), and acclimatized for one week prior to use. The rats were housed in metal cages in the metabolic laboratory with uniform temperature of $22 \pm 2^\circ\text{C}$, with humidity of $50 \pm 10\%$ and 12-hrs light-dark cycle. Rodent laboratory chow pellets and tap water were supplied *ad libitum*. All animal handling procedures (used in this experiment) were performed in strict compliance with the P. R. China legislation for the use and care of laboratory animals, the guidelines established by Institute for Experimental Animals of TangShan Teacher's College, and were approved by the local ethics committee for animal experiments.

Animals of grouping

A total of 40 rats were divided into four groups equally based on body weight: namely, the control (C) group, low-treated (LT) group, medium-treated (MT) group, and high-treated (HT) group. The treated (LT, MT and HT) groups received various doses of PGP (100, 200 and 400 mg/kg body weight, respectively), and the control group received the same volume of drinking water. PGP were dissolved in 2.0 mL of drinking water. The treatments were administered orally and daily for 30 days. After each treatment, all groups of the rats were allowed to rest for 30 min and were forced to swim for 20 min to become accustomed to swimming.

Forced swimming test

After 30 days, forced swimming test was carried out in an acrylic plastic pool (90 cm × 45 cm × 45 cm) filled with water ($28 \pm 1^\circ\text{C}$) to a depth of 37 cm. A steel washer (7% of body weight), was loaded on the tail root of each rat. The swimming period was regarded as the time spent by the rats floating in the water with struggling and making necessary movements until exhausting its strength. The rats were considered exhausted when they failed to rise to the surface to breathe after 10 s (Bing and Zhaobao, 2010; Li et al., 2013), and the exhaustive swimming time was measured.

Measurement of some biochemical parameters related to fatigue

The rats were anesthetized with ketamine hydrochloride (20 mg/kg body weight), and sacrificed immediately after the forced swimming test. Blood samples were collected from the abdominal aorta for assaying of BLA, BUN and hemoglobin concentrations. After the blood was collected, the liver and gastrocnemius muscle were immediately dissected, frozen in liquid nitrogen, and kept at -80°C until analysis of glycogen concentrations was performed. The BLA, BUN, hemoglobin, liver glycogen and muscle glycogen concentrations were determined using commercial diagnostic kits following the manufacturer's instructions. BLA concentrations were determined by the colorimetric method and 530 nm was chosen as the test wavelength. BUN concentrations were determined by the colorimetric method and 520 nm was chosen as the test wavelength. Hemoglobin concentrations were determined by the cyanmethemoglobin method and 540 nm was chosen as the test wavelength (Von Kompen and Zijlstra, 1961). Glycogen concentrations were determined by the anthrone reagent method and 620 nm was chosen as the test wavelength.

Statistical analysis

Statistical analysis was performed using Student's t-test and one way analysis of variance (one way-ANOVA). The accepted level of significance was preset as $p < 0.05$. Values are expressed as means \pm SD.

Results and discussion**Effects of PGP on exhaustive swimming time**

Forced swimming test was employed in this study to evaluate the effects of PGP supplementation on physical fatigue. It is commonly accepted that swimming is an experimental exercise model (Ma et al., 2008). Other methods of forced exercise such as the motor driven treadmill or wheel can cause animal injury and may not be routinely acceptable (Wu et al., 1998). The length of exhaustive swimming time indicates the degree of fatigue. As shown in Figure 1, the exhaustive swimming time of LT, MT and HT groups were significantly longer than that of the C group ($p < 0.05$), and the increase ratios were 30.48%, 38.36% and 49.44%, respectively. The results demonstrated that PGP could elevate the exercise tolerance, which suggested that PGP possesses an anti-fatigue effect. To explore the mechanisms, some biochemical parameters related to fatigue, such as BLA, BUN, hemoglobin, hepatic glycogen and muscle glycogen were determined in the rats after forced swimming test.

Effects of PGP on blood lactic acid

Blood lactic acid (BLA), is the glycolysis product of carbohydrate under an anaerobic condition, and glycolysis is the main energy source for fierce exercise in a short time (Wang et al., 2006; Zhang et al., 2010; Chuanlong and Xiaoxia, 2011). Therefore, BLA is closely related to workload intensity and is one of the important indicators for determining the intensity of the exercise or the degree of fatigue. As shown in Figure 2, the BLA concentrations of LT, MT and HT groups were significantly lower than that of the C group ($p < 0.05$), and the decrease ratios

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were 32.08%, 43.78% and 52.24%, respectively. The results demonstrated that PGP could effectively retard and lower the BLA produced, and postpone the appearance of fatigue.

Effects of PGP on blood urea nitrogen

Urea is formed in the liver as the end product of protein metabolism (Chen et al., 2013). During digestion, protein is broken down into small peptides and amino acids. The amino acid nitrogen is removed as NH_4^+ , while the rest of the molecule is used to produce energy or other substances needed by the cell (Ding et al., 2011; You et al., 2011). Circulating ammonia is taken up by the liver and most of it detoxified in this tissue through the urea cycle. Thus blood urea nitrogen (BUN), which is a product of energy metabolism, is another sensitive index of fatigue status (Cao et al., 2012). As shown in Figure 3, the BUN concentrations of MT and HT groups were significantly lower than that of the C group

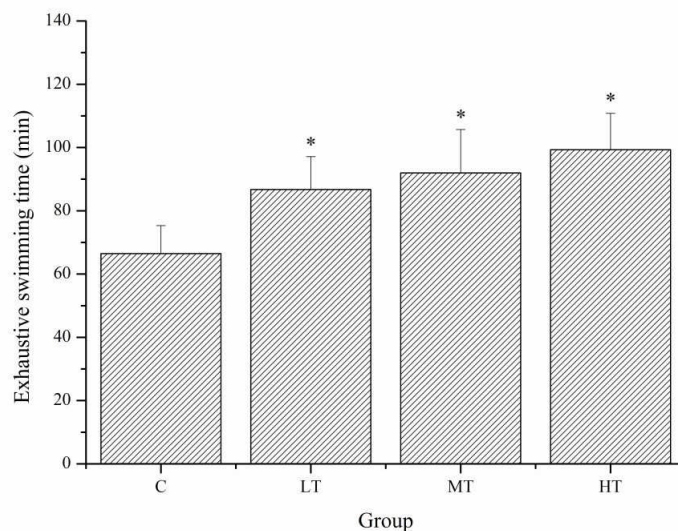


Figure 1: Effects of PGP on the exhaustive swimming time. Values are expressed as means \pm SD of ten. * $p < 0.05$, compared with control (C) group.

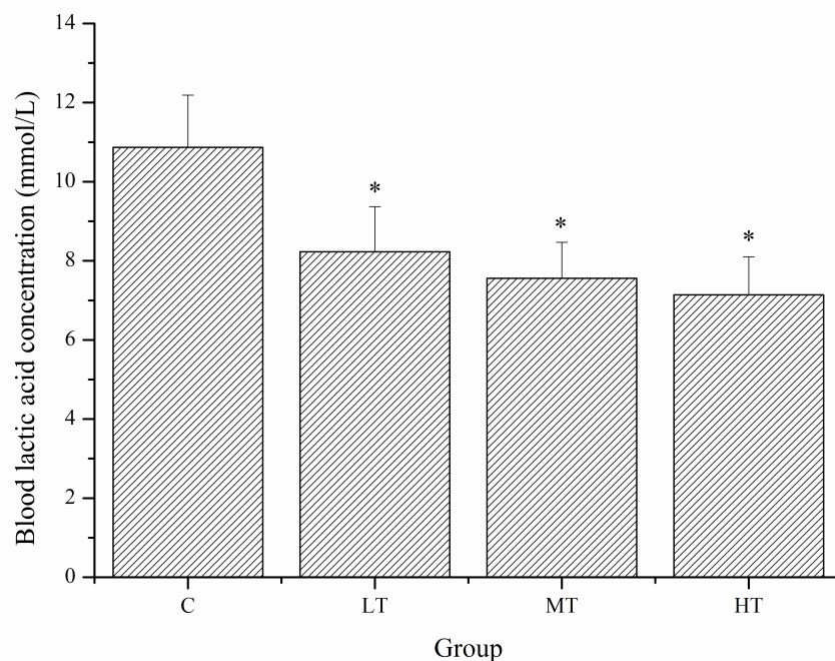


Figure 2: Effects of PGP on the blood lactic acid. Values are expressed as means \pm SD of ten. * $p < 0.05$, compared with control (C) group.

($p < 0.05$), and the decrease ratios were 15.06% and 23.89%, respectively. The results demonstrated that PGP could reduce the decomposition of nitrogenous substances in the body and improved endurance capacity during exercise.

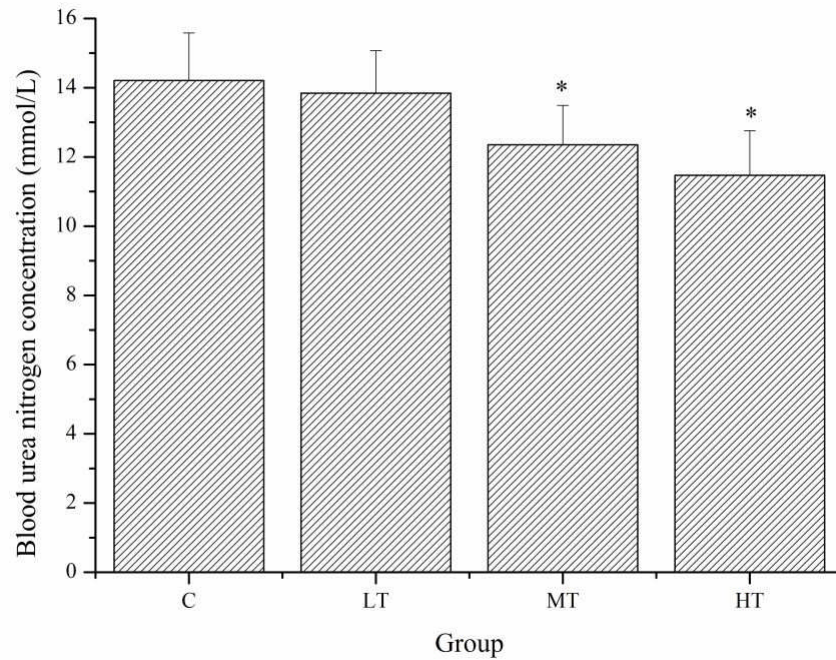


Figure 3: Effects of PGP on the blood urea nitrogen. Values are expressed as means \pm SD of ten. * $p < 0.05$, compared with control (C) group.

Effects of PGP on hemoglobin

Hemoglobin is the main component of erythrocyte. Its main function is to serve as the carrier for the erythrocyte to transport oxygen and partial carbon dioxide. Hemoglobin also has the effect on maintaining the body fluid's acid-alkali balance (Cao et al., 2009; Ding et al., 2009). Therefore, it can directly affect the substance metabolism and the energy metabolism in the body and, in turn, affect body function and exercise ability of the human body, the exercise's loading capacity, and fatigue. Thus hemoglobin is one of the indicators that reflect the degree of recovery from fatigue after exercise (Wang et al., 2006). As shown in Figure 4, the hemoglobin concentrations of LT, MT and HT groups were significantly higher than that of the C group ($p < 0.05$), and the increase ratios were 21.67%, 26.71% and 34.89%, respectively. The results demonstrated that PGP could influence the supply of oxygen to tissues by hemoglobin, which is another confirmation that PGP has an anti-fatigue effect.

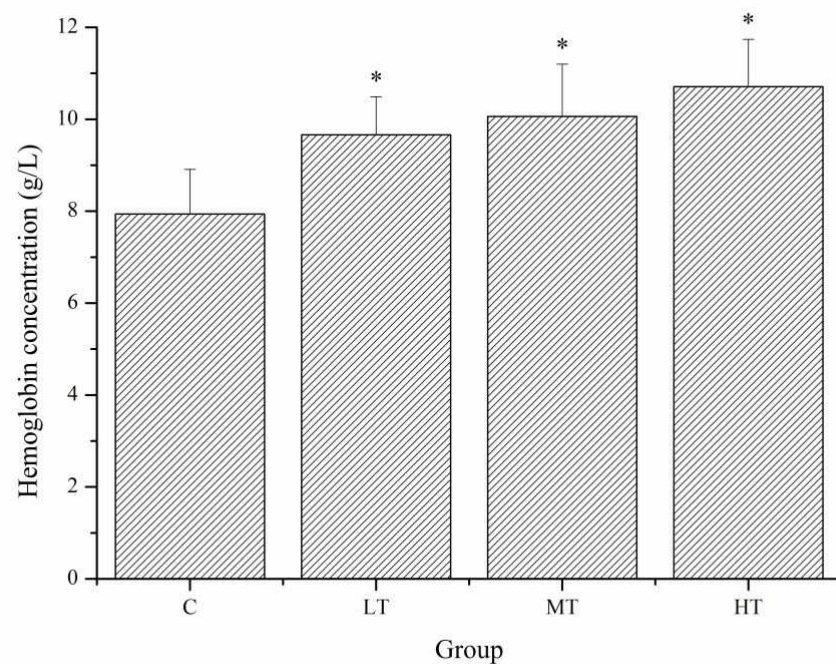


Figure 4: Effects of PGP on the hemoglobin. Values are expressed as means \pm SD of ten. * $p < 0.05$, compared with control (C) group.

Effects of PGP on liver glycogen and muscle glycogen

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 (Shang et al., 2009; Prasad and Khanum, 2012; Liu et al., 2013). Therefore, glycogen is a sensitive index to test fatigue. As shown in Figure 5, the liver glycogen concentrations of LT, MT and HT groups were significantly higher than that of the C group ($p < 0.05$), and the increase ratios were 35.48%, 55.69% and 67.20%, respectively. The muscle glycogen concentrations of LT, MT and HT groups were significantly higher than that of the C group ($P < 0.05$), and the increase ratios were 42.52%, 59.84% and 54.33%, respectively. The results demonstrated that the anti-fatigue effects of PGP may be related to the improvement in the metabolic control of exercise and the activation of energy metabolism.

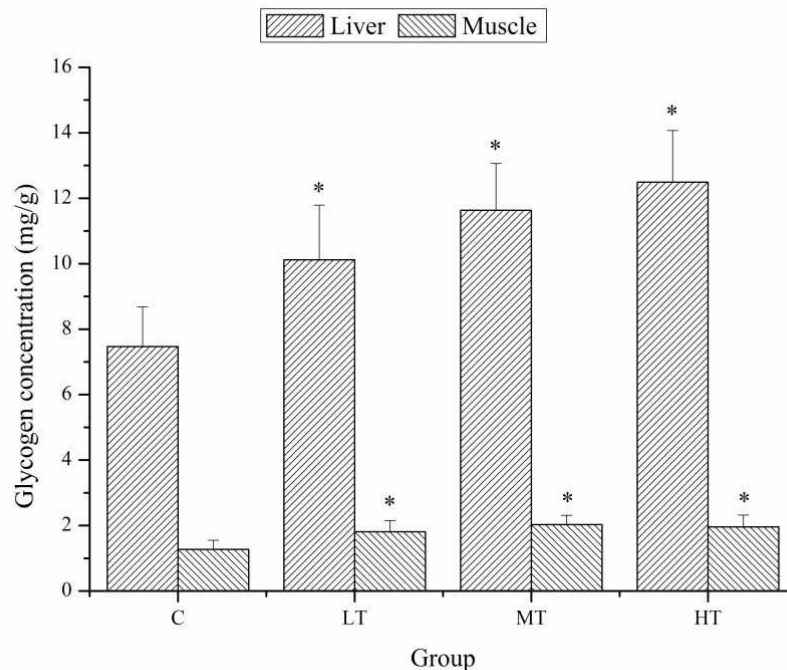


Figure 5: Effects of PGP on the liver glycogen and muscle glycogen. Values are expressed as means \pm SD of ten. * $p < 0.05$, compared with control (C) group

Conclusion

The present study clearly showed that PGP possessed an anti-fatigue effect, which could extend the exhaustive swimming time of the rats and elevate the exercise tolerance, as well as decrease the BLA and BUN concentrations, and increase the hemoglobin, liver glycogen and muscle glycogen concentrations. However, further research is needed in order to elucidate the more exact mechanism of the effects of PGP on physical fatigue.

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