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Full Length Research Paper

Effects of Gypenosides from *Gynostemma pentaphyllum* supplementation on exercise-induced fatigue in mice

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This study was designed to determine the effect of Gypenosides from *Gynostemma Pentaphyllum* (GGP) on exercise-induced fatigue in mice. Forty-eight mice were studied by being divided into three group (n = 16 per group) included the normal control group (NC), the low dose GGP group (LG) and the high dose GGP group (HG). The GGP groups were first administered different doses of GGP (50 and 100 mg/kg), while the NC group were force administered 1% carboxymethylcellulose for 28 days. The GGP groups showed a significant increase in swimming time to exhaustion as compared to the control group. Blood lactate concentration of the GGP groups was significantly lower and blood glucose concentration of the GGP groups was significantly higher than that in the NC group. In conclusion, GGP may have beneficial effects on exercise-induced fatigue. GGP Supplementation can extend the swimming time for the mice, effectively delay the lowering of glucose in the blood, and prevent the increase in lactate.

Key words: Gypenosides from *Gynostemma pentaphyllum*, fatigue, exercise, swimming endurance capacity.

INTRODUCTION

Exercise-induced fatigue has been attributed to the following factors. First, myoglobin and an energy metabolic system coenzyme leak out into the blood from cells and tissues damaged by exercise, and destruction of red blood cells occurs. Second, exercise promotes consumption of energy sources such as glycogen by mobilizing internal energy metabolism to the maximum and using and depleting the energy source. Third, through these processes, exercise causes the production and accumulation of products of metabolism, such as lactic acid, in the body (Grenhaff and Timmonns, 1998; Pedersen et al., 2004; Ikeuchi et al., 2006). Exercise-Induced Fatigue can be recovered by being supplemented energetic substance, releasing metabolic production and being administrated tonics, but these bring harms to the body even though retarding the fatigue (Li and Wei, 2005). In addition, some of the drugs are forbidden by International Olympic Committee. During seeking for safe and effective anti-fatigue methods, the specialty of oriental medicinal herb has drawn the attention of scholars in the world (Lu et al., 2009).

Gynostemma pentaphyllum (botanical name) or *Jiaogulan* (Chinese name) is an herbaceous vin plant of the cucurbitaceous family and distributes naturally in shaded and humid places. And it is an oriental medicinal herb for heat clearing, detoxification and expectorant for relieving cough in southern China, Japan, India, and Korea (Megalli et al., 2005; Kuwahara et al., 1989). The significance of *G. pentaphyllum* in pharmacology and

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Abbreviations: GGP, Gypenosides from *Gynostemma pentaphyllum*; **NC,** normal control group; **LG,** low dose GGP group; **HG,** high dose GGP group.

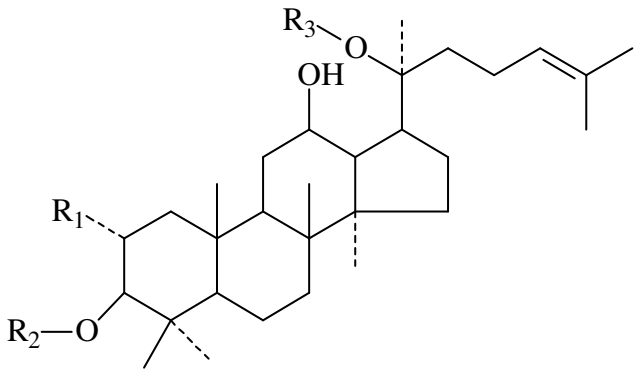

Figure 1. General structure of Dammarane -Type Gypenosides. Gypenoside consists of both the hydrophobic sapogenin part and the hydrophilic sugar part in the molecule (where R1 and R2 = glucose, rhamnose; R3 = glucose, xylose).

Table 1. The composition of basal diet.

Ingredients	Content (%)	Ingredients	Content (%)
Corn starch	62.00	L-cystine	0.50
Casein	16.00	Mineral mix	3.50
Sucrose	8.25	Vitamin mix	1.00
Soybean oil	3.50	Choline chloride	0.50
a-cellulose	4.75		

Minerals added per kg diet: Ca, 5000 mg; P, 1761 mg; K, 3400 mg; Na, 1091 mg; Cl, 1852 mg; S, 300 mg; Mg 753 mg; Fe, 100 mg; Mn, 75 mg; Cu, 10 mg; Zn, 30 mg; I, 0.5 mg; F, 0.8 mg; Mo, 0.2 mg; Co, 0.25 mg. Vitamins added per kg diet: Vitamin A, 13000 IU; Vitamin D, 1000 IU; Vitamin E, 40 IU; Vitamin K, 3 mg; Vitamin B1, 8 mg; Vitamin B2, 10 mg; Vitamin B6, 6 mg; Vitamin B12, 0.02 mg; Niacin, 45 mg; Pantothenic acid, 17 mg; Biotin, 0.2 mg; Vitamin C, 150 mg.

health has been investigated, and the results show that *G. pentaphyllum* possesses important biological functions, such as inhibiting the propagation of cancer cells, antiaging, cholesterol-lowering, immunopotentiating, antioxidant, hypoglycemic, antiulce-ration and antidiabetic effects (Zhu et al., 2001; Norberg et al., 2004; Chang et al., 2005; Razmovski-Naumovsk et al., 2005). Phytochemical studies of *Gynostemma pentaphyllum* have identified approximately 90 dammarane - type saponin glycosides, known as gypenosides, which are responsible for its pharmacological activities (Yin et al., 2004).

A general structure of dammarane - type gypenoside is illustrated in Figure 1 (Megalli et al., 2005). The chemical structure of gypenosides closely resembles that of ginsenosides found in panax ginseng (Chang et al., 2005). However, no detailed study has been reported on the effect of exercise-induced fatigue of gypenosides from *G. pentaphyllum* (GGP). Therefore, in the present work, we investigated exercise-induced fatigue by administering GGP to mice and then subjecting the animals to exercise in the form of swimming.

MATERIALS AND METHODS

Materials

Purified gypenosides from *gynostemma pentaphyllum* (GGP) was provided by Shanghai Boyun Biotech. Co., Ltd, PRC as a reference sample. Crude GGP containing more than 90% gypenosides was used for the experiments and was derived from a capsule formulation consisting of purified extract diluted to 30% with excipient (Shanxi Haoyang Biotech. Co., Ltd, PRC). The capsule formulation was placed in 90% ethanol, thoroughly mixed with a magnetic stirrer then filtered twice using filter paper and evaporated at 50°C down to a solid in a Buchi Rotavapor R114 over 24 h (Megalli et al., 2006). The Crude GGP was then dried in a vacuum oven and kept desiccated in a bell jar with silica gel. The resulting crude GGP contained approximately 90% gypenosides.

Animals

Male Kunming mice (4 weeks old, 20 ± 2 g) were obtained from the Animal Department of Top Vocational Institute of Information and Technology of Shaoxing. All animals were maintained in separated cages (38 × 60 × 30 cm, 8 mice/cage) with tap water. Under the conditions of 21 ± 1, and 50 - 60% relative humidity, they were allowed free access to basal diet (purchased from Zhejiang Research Animal Center, Hangzhou, China) and water and kept on 12 h light/dark cycles. The ingredient and nutrient compositions of the basal diet are shown in Table 1. All experiments were performed in accordance with the institutional ethical guideline (World Health Organization (WHO) Chronicle, 1985).

Evaluation of the swimming endurance capacity of mice

The mice were allowed to adapt to the laboratory housing condition for at least 1 week. Forty-eight mice were divided into three groups (n = 16 per group) equally based on body weight including the normal control group (NC), the low dose GGP group (LG) and the high dose GGP group (HG). Crude GGP samples were well mixed in 1% carboxymethylcellulose. The mice in the LG and HG groups were force administered different doses of GGP (50 and 100 mg/kg) in a volume of 1.0 mL using oral gavage via a curved feeding needle at 9:30 once daily for 28 days. The mice in the NC group were force administered with the same volume of 1% carboxymethylcellulose.

The mice were submitted to weekly swimming exercise supporting constant loads (lead fish sinkers, attached to the tail) corresponding to 5% of their body weight. The mice where assessed to be fatigued when they failed to rise to the surface of the water to breathe within 5 s (Ikeuchi et al., 2005; Sun et al., 2006).

The swimming exercise was carried out in a acrylic plastic pool (60 × 40 × 40 cm), filled with water to a depth of 30 cm. The temperature of the water was maintained at 30 ± 1°C. To avoid circadian variations in physical activity, swimming exercise was performed between 11:00 and 17:00 (Ikeuchi et al., 2006).

Assay for blood lactate and glucose

After a period of 28 days, eight mice were taken out from each group for blood lactate and glucose analyses. Each of the mice had

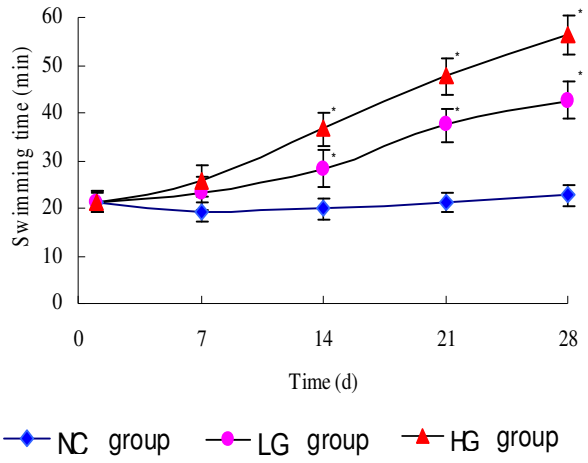

Figure 2. Effect of GGP on swimming exercise in mice. Values are mean ± SD of 16 mice per group. * P < 0.05 vs. NC group.

a weight attached (5% body weight) to the tail for the duration of the swim-to-exercise (15 min). Blood samples were collected 7 times from the tail before the beginning and at 5 min intervals during swimming exercise, as well as 10, 30 and 60 min break after swimming exercise (Ikeuchi et al., 2006). To avoid blood dilution with residual water at the tail of the animal, the mice were quickly dried with a towel immediately before blood collection. The mice were immediately returned to the acrylic plastic pool after blood sampling.

Blood lactate levels were determined by using a commercial diagnostic kit obtained from Jiancheng Diagnostic Co. (Nanjing, China). Blood glucose levels were determined by using glucose analyzer and strips obtained from Roche Diagnostic Co. (USA).

Statistical analysis

Data were examined for equal variance and normal distribution prior to statistical analysis. Mean values were compared by student's t-test or analysis of variance (ANOVA) by using the SPSS 13.0 software (Spss Inc. Chicago, IL, USA). A significance level of 5% was adopted for all comparisons.

RESULTS AND DISCUSSION

Effects of GGP on swimming exercise of mice

The anti-fatigue effect of GGP was investigated by using swimming exercise. It is commonly accepted that swimming is an experimental exercise model (Feng et al., 2009). Before embarking on the experiment, all the groups had no significant difference in swimming times (P > 0.05).

A significant (P < 0.05) increase in swimming times was detected in the LG and HG groups as compared to the NC group from 14 days onward. The results indicated that different doses of GGP supplementation had significant effect on endurance capacity of mice and the dosage of 100 mg/kg was more effective than that of 50 mg/kg. The results were shown in Figure 2.

Effects of GGP on blood lactate in exercising mice

Blood lactate is the glycolysis product of carbohydrate under an anaerobic condition, and glycolysis is the main energy source for intense exercise in a short time. Therefore, the blood lactate is one of the important indicators for judging the degree of fatigue (Yu et al., 2008; Tang et al., 2009). The blood lactate concentrations of mice were measured as described in the methods. The results were shown in Figure 3. It was found that the blood lactate concentration of each group had no significant difference (P > 0.05) before swimming. However, after swimming for 15 min and break for 30 min, the blood lactate concentration of the LG and HG groups were significantly lower than that of the NC group (P < 0.05).

In this study, the results showed that different doses of GGP supplementation can effectively retard and lower the blood lactate produced after swimming, postpone the appearance of fatigue and accelerate the recovery from fatigue.

Effects of GGP on blood glucose in exercising mice

Homeostasis of blood glucose is important for the prolongation of endurance exercise (Ahlborg and Felig, 1982; Abe et al., 1995; Astorino et al., 2000). The blood glucose concentrations of mice were measured as described in the methods. The results were shown in Figure 4.

It was found that the blood glucose concentration of each group had no significant difference (P > 0.05) before swimming. However, after swimming for 15 min, the blood glucose concentration of the LG and HG groups were significantly higher than that of the NC group (P < 0.05). In this study, these results indicate that the prolongation of the swimming times seen in mice sup-plying different doses of GGP must be brought about by an improvement in the physiological function or metabolic control of exercise as well as by an activation of energy metabolism.

Conclusion

In conclusion, our data suggest that GGP has beneficial effects on exercise-induced fatigue. GGP supplementation can extend the swimming time for the mice, effectively delay the lowering of glucose in the blood, and prevent the increase in lactate. However, comprehensive chemical and pharmacological research is required to determine the mechanism by which GGP affects exercise-induced fatigue.

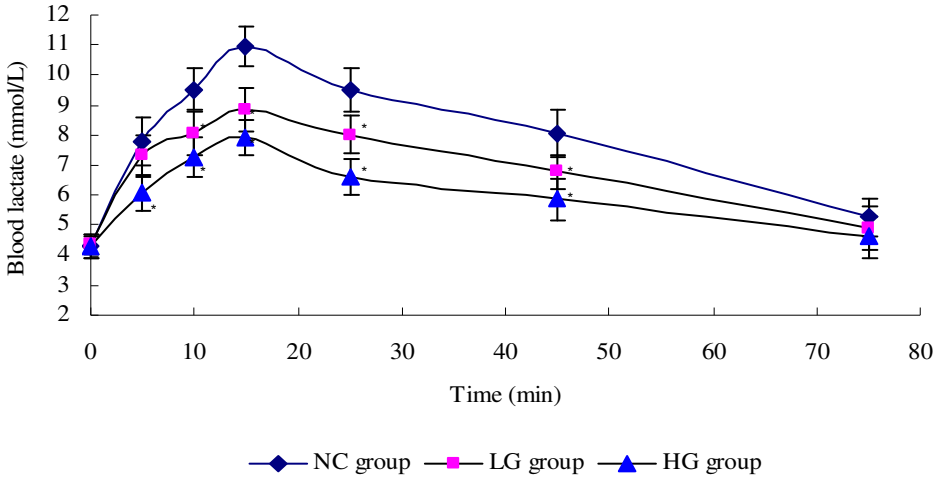

Figure 3. Effects of GGP on blood lactate in exercising mice.
Values are mean ± SD of 8 mice per group. * P < 0.05 vs. NC group.

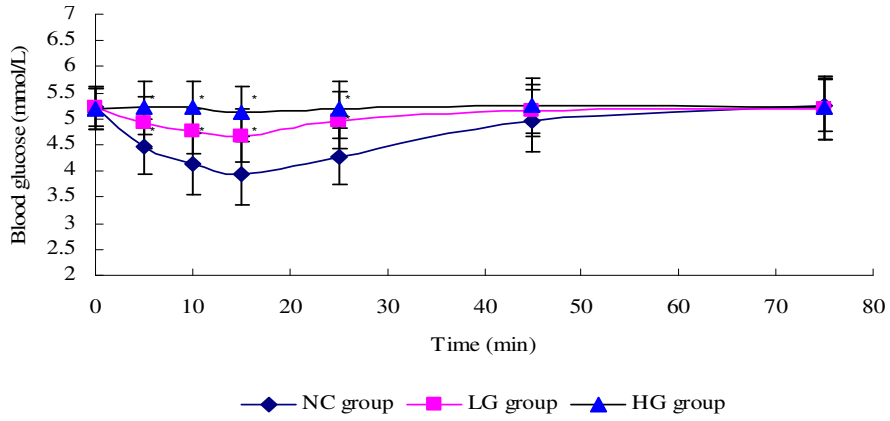

Figure 4. Effects of GGP on blood glucose in exercising mice.
Values are mean ± SD of 8 mice per group. * P < 0.05 vs. NC group.

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