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## Effects of *Ganoderma lucidum* Polysaccharides on Exercise-induced Fatigue in Mice

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### ABSTRACT

*Ganoderma lucidum*, a popular medicinal mushroom, has been widely used as a herbal medicine for promoting health and longevity in China and other oriental countries. *Ganoderma lucidum* polysaccharides (GL-PS) are its main active components and have a lot of pharmaceutical activities. The present study was designed to determine the effects of GL-PS on exercise-induced fatigue in male Kunming mice. The mice were divided into four groups (three GL-PS administered groups and the control group). The control group were administered with distilled water and GL-PS administered groups were administered with GL-PS (50, 100 and 200 mg kg<sup>-1</sup> b.wt.). After 28 days, anti-fatigue effects was evaluated using a forced swimming test, along with the determination of Blood Lactic Acid (BLA), Serum Urea Nitrogen (SUN), liver glycogen, Superoxide Dismutase (SOD) and glutathione peroxidase (GSH-Px) contents. The results suggested that GL-PS could extend the swimming time to exhaustion of the mice, as well as increase the liver glycogen, SOD and GSH-Px contents, while decrease the blood lactic acid and serum urea nitrogen contents. Therefore, GL-PS had anti-fatigue effects.

**Key words:** *Ganoderma lucidum* polysaccharides, exercise-induced fatigue, mice, forced swimming test

### INTRODUCTION

*Ganoderma lucidum* (Leyss. ex Fr.) Karst.), a popular medicinal mushroom, is a basidiomycete belonging to the polyporaceae. Its fruiting body is more commonly known as "Lingzhi" in China (Zhang *et al.*, 2003; Yang *et al.*, 2004). *G. lucidum* has been widely used as a herbal medicine for promoting health and longevity in China and other oriental countries, such as Japan and Korea (Lin and Zhang, 2004). In ancient China, it was regarded as "herbal medicine from heaven" because it was thought to be able to make a person long live or make a dead person back to life. Earlier investigation have demonstrated that *Ganoderma lucidum* has a large variety of pharmacological functions and plays an important role in preventing and treating various diseases, such as chronic hepatopathy, gastric ulcer, obesity, diabetes, hyperlipidemia, Inflammation, thrombosis, asthma, aging and neoplasia, etc. (Gao *et al.*, 2002; Chien *et al.*, 2004; Ni *et al.*, 2007;

Wang *et al.*, 2009; Balazs, 2010; Kumaran *et al.*, 2011; Joseph *et al.*, 2011). Previous evidences showed that polysaccharide and triterpene are two major categories of compounds purified from *G. lucidum*. It has been shown that *G. lucidum* polysaccharides (GL-PS) have anti-oxidant, hypoglycemic, anti-inflammatory, anti-tumor and immunomodulatory activities (Lin and Zhang, 2004; Liu *et al.*, 2010; Li *et al.*, 2011; Joseph *et al.*, 2011). However, little information about the anti-fatigue effects of GL-PS is currently known. Therefore, the present study was designed to determine the effects of GL-PS on exercise-induced fatigue in mice and aimed to provide experimental basis for further study of GL-PS.

## MATERIALS AND METHODS

**Chemicals and reagents:** The study was conducted in Laboratory of Biochemistry of Zhejiang Police College between November 2010 and June 2011. Analytical reagent grade chemicals and double distilled water were used to prepare all solutions. The reagent kits for determination of Blood Lactic Acid (BLA), Serum Urea Nitrogen (SUN), liver glycogen, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were purchased from Nanjing Jiancheng Bioengineering Institute, Nanjing, China.

**Experimental animals:** Four to five week-old male Kunming mice weighing 18-20 g, offered by Zhejiang Animal Husbandry Center (Hangzhou, China), were used in the experiments. The animals had access to a standard commercial diet and water *ad libitum* and were kept in rooms maintained at 22±1°C with a 12 h light/12 h dark cycle throughout the experiments. All animal handling procedures were performed in strict accordance with the China legislation the use and care of laboratory animals, with the guidelines established by Institute for Experimental Animals of Zhejiang Police College and were approved by the College committee for animal experiments.

**Preparation of *G. lucidum* polysaccharides:** The fruiting bodies of *G. lucidum* were purchased from a local medicine shop (Hangzhou, China) and identified by Professor Zhang ML, Zhejiang Gongshang University. Voucher specimens were preserved in the Herbarium of the Zhejiang Institute of Botany. *G. lucidum* polysaccharides (GL-PS) were prepared by the method as described earlier with slight modification (XiaoPing *et al.*, 2009). In brief, the dried fruiting bodies of *G. lucidum* were ground to fine powder and powders were defatted with petroleum ether and extracted with double distilled water at 80°C for 8-10 h in several batches. The extract were combined, filtered and concentrated to about one third of the original volume and chilled ethanol about five times the original volume was added and kept at 4°C for 48 h. The precipitate was collected after centrifugation, redissolved in distilled water and treated with Sevag's reagent several times to remove protein and then dialyzed against deionised water for 48 h at 4°C. The GL-PS were again precipitated with ethanol and the precipitate thus obtained was lyophilized. GL-PS was dissolved in distilled water and stored at 4°C before use.

**Grouping of animals:** Total 80 male Kunming mice were divided into four groups equally based on body weight after one week adaptation: The first group designated as control group were administered with distilled water by gavage per day for 28 days. The second group designated as low dose group were administered with GL-PS of 50 mg kg<sup>-1</sup> b.wt. by gavage per day

for 28 days. The third group designated as middle-dose group were administered with GL-PS of 100 mg kg<sup>-1</sup> b.wt. by gavage per day for 28 days. The fourth group designated as high-dose group were administered with GL-PS of 200 mg kg<sup>-1</sup> b.wt. by gavage per day for 28 days. The mice were trained to swim for 20 min twice a week to accustomize to swimming. After 28 days, the mice were fasted overnight before forced swimming test.

**Forced swimming test:** After 28 days, 10 mice were taken out from each group for the forced swimming test, which was used as described previously with some modifications (Cai *et al.*, 2010; Tang *et al.*, 2008). Briefly, 30 min after the last administration, the mice were placed individually in a swimming pool (50×40×50 cm) with 30 cm depth of water maintained at 25±0.5°C. A tin wire (7% of body weight) was loaded on the tail root of the mouse. Swimming time to exhaustion was considered as time spent by the mice when they were floating in water, struggling and only making those movements necessary to keep their heads above water until exhausting their strength and drowning. The mice were determined to be exhausted when they sunk into the water and couldn't rise to the surface of water within a 10 sec period.

**Analysis of blood biochemical parameters:** After 28 days, the other 10 mice were taken out from each group for analyses of blood biochemical parameters. 30 min after the last administration, the mice were forced to swim in the swimming pool (weight-unloaded) for 90 min. Rested for 60 min, the mice were anesthetized with pento-barbital sodium 5 (Li and Li, 2009). The blood samples were collected in heparinized tubes by heart puncture at mice. Plasma was prepared by centrifugation at 900×g, 4°C for 10 min and stored at -70°C in a deep-freezer 4 (Jung *et al.*, 2004). The BLA and SUN contents were tested following the recommended procedures provided by the kits.

**Analysis of liver glycogen and antioxidant enzyme:** Immediately after the blood had been collected, liver were quickly dissected out, frozen in liquid nitrogen and kept at -70°C until analysis for glycogen and antioxidant enzyme 4 (Jung *et al.*, 2004). The liver glycogen, SOD and GSH-Px contents were tested following the recommended procedures provided by the kits.

**Statistical analysis:** The data were expressed as Mean±SD based on the indicated number in the experiment. All analyses of data were done with the statistical package for social sciences (version 11.0; SPSS, Chicago, IL, USA). The results were analyzed using 1 way analysis of variance followed by student t-test for comparison between different treatment groups. Statistical significance was set at p<0.05.

## RESULTS

**GL-PS lengthens the swimming time to exhaustion of mice:** The effects of GL-PS on swimming time to exhaustion of mice are shown in Fig. 1. The swimming time to exhaustion of mice in the second, third and fourth groups were significantly prolonged compared to the first group (p<0.05) and the increase ratios were 22.7, 48.6 and 67.8%, respectively.

**GL-PS decreases blood lactic acid contents during exercise:** The effects of GL-PS on blood lactic acid of mice are shown in Fig. 2. The BLA contents of mice in

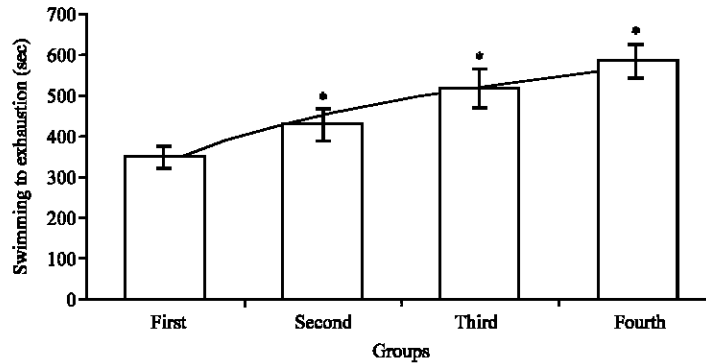


Fig. 1: The effects of GL-PS on swimming time to exhaustion of mice. The data were expressed as Means±SD (n = 10 per group). \*p<0.05 when compared to the first group

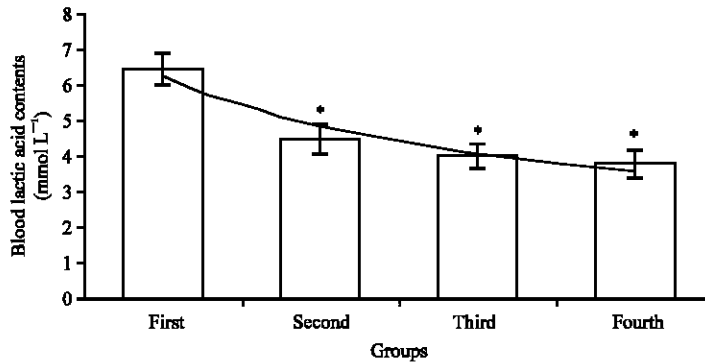


Fig. 2: The effects of GL-PS on blood lactic acid of mice. The data were expressed as Means±SD (n = 10 per group). \*p<0.05 when compared to the first group

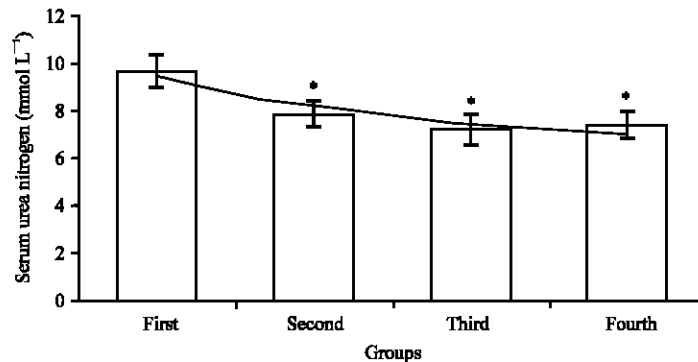


Fig. 3: The effects of GL-PS on serum urea nitrogen of mice. The data were expressed as Means±SD (n = 10 per group). \*p<0.05 when compared to the first group

the second, third and fourth groups were significantly lower compared to the first group (p<0.05) and the decrease ratios were 57.6, 60.8 and 68.8%, respectively.

**GL-PS decreases serum urea nitrogen contents during exercise:** The effects of GL-PS on Serum Urea Nitrogen (SUN) of mice are shown in Fig. 3. The SUN contents of mice in the second, third and fourth groups were significantly lower compared to the first group (p<0.05) and the decrease ratios were 23.5, 34.1 and 30.6%, respectively.

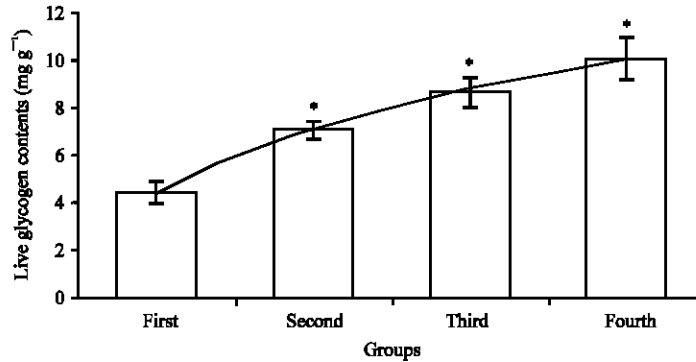


Fig. 4: The effects of GL-PS on liver glycogen of mice. The data were expressed as Means±SD (n = 10 per group). \*p<0.05 when compared to the first group

Table 1: The Effects of GL-PS on SOD and GSH-Px of mice

Groups	SOD (IU mg <sup>-1</sup> pro)	GSH-Px (IU mg <sup>-1</sup> pro)
First	106.83±90.41	4.96±0.87
Second	139.26±11.28*	6.82±0.63*
Third	157.34±10.37*	9.36±0.81*
Fourth	168.29±13.89*	12.27±1.06*

The data were expressed as Means±SD (n = 10 per group). \*p<0.05 when compared to the first group

**GL-PS increases liver glycogen contents during exercise:** The effects of GL-PS on liver glycogen of mice are shown in Fig. 4. The liver glycogen contents of mice in the second, third and fourth groups were significantly higher compared to the first groups (p<0.05) and the increase ratios were 59.2, 95.1 and 125.9%, respectively.

**GL-PS increases SOD and GSH-Px contents during exercise:** The effects of GL-PS on SOD and GSH-Px of mice are shown in Table 1. The SOD and GSH-Px contents of mice in the second, third and fourth groups were significantly higher compared to the first group (p<0.05).

## DISCUSSION

The Forced Swimming Test (FST) was employed in this study to evaluate the anti-fatigue effects of GL-PS. It is commonly accepted that swimming is an experimental exercise model (Shin *et al.*, 2004; Ikeuchi *et al.*, 2006; Jung *et al.*, 2007). Some weights (tin wires, lead pieces and iron wires, etc.) were added to the chests or tails of the animals to decrease the workload and shorten the swimming time of the mice (Matsumoto *et al.*, 1996). In this study, the mice had a weight attached 7% body weight in the duration of the swimming to exhaustion and the results showed that GL-PS could extend the swimming time to exhaustion, which indicated that GL-PS had anti-fatigue effects and could elevate the exercise tolerance.

It is well known that the muscle produces plenty of lactic acid when it obtains enough energy from anaerobic glycolysis almost at the same time when doing strenuous exercise. The increased lactic acid production results in decreasing the internal pH value, which may lead to impairment of muscle contraction and harm organs. So Blood Lactic Acid (BLA) can be regarded as one of the main indicators to evaluate degree of fatigue (Cao *et al.*, 2009; Fu *et al.*, 2010; You *et al.*, 2011). The obtained results of present study indicated that GL-PS could effectively retard and lower the BLA produced, postpone the appearance of fatigue.

Serum urea nitrogen, the product of energy metabolism when moving, is a sensitive index to evaluate the bearing capability when human bodies suffer from a physical load (Huang *et al.*, 2011). Many reports have pointed out that SUN increases with the increase of physical loads and the worse human bodies can be adapted to the physical loads, the more obviously the increase of SUN is (Zhang *et al.*, 2006; Tang *et al.*, 2008). The obtained results of present study indicated that GL-PS could reduce catabolic decomposition of protein for energy and enhance exercise tolerance.

Glycogen is the important resource of energy during exercise, the contents of which directly lead to degree of physical endurance. After strenuous exercise, with the increase of exhausted muscle glycogen, liver glycogen will be released to maintain the blood glucose in the physiologic range. So liver glycogen is another main indicator to evaluate degree of fatigue (Prasad and Khanum, 2012; Zhang *et al.*, 2010). The obtained results of present study indicated that GL-PS might increase tissue glycogen contents of mice post exercise by improving glycogen reserve, or by reducing the glycogen consumption during exercise, or both. However, the detailed mechanism of this phenomenon is not clear and needs further study.

It is well documented that exhaustion exercise-induced oxidative stress could produce reactive oxygen species, such as superoxide anion radical, hydroxyl radical, or hydrogen peroxide. Intracellular antioxidant enzymes, including SOD and GSH-Px, could reduce oxidative stress mediated body fatigue (Tharakan *et al.*, 2005; Wang *et al.*, 2008). The obtained results of present study indicated that GL-PS could promote increases in the activities of these antioxidant enzymes, again supporting that GL-PS has anti-fatigue effects.

## CONCLUSION

In conclusion, the present study has shown that GL-PS had anti-fatigue effects, which could extend the swimming time to exhaustion of the mice, as well as increase the liver glycogen, SOD and GSH-Px contents, while decrease the blood lactic acid and serum urea nitrogen contents. The results provide an important basis for developing the GL-PS as an anti-fatigue compound. However, further research needs to be carried out to evaluate its anti-fatigue activity on humans and its anti-fatigue mechanism(s) at the cellular and molecular levels.

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