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# Evaluation of Antioxidant and Anti-Fatigue Activities of *Ganoderma lucidum* Polysaccharides

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**Abstract:** This study was designed to evaluate the antioxidant and anti-fatigue activities of *Ganoderma lucidum* Polysaccharides (Gl-PS). *In vitro* antioxidant activities of Gl-PS were investigated through DPPH and superoxide radical scavenging activities. *In vivo* anti-fatigue activity of Gl-PS was investigated through forced swimming test of mice. Results showed that Gl-PS had strong scavenging activity to DPPH and superoxide radical. *In vivo* experimental studies showed that Gl-PS had anti-fatigue activity which could evidently extend exhaustive swimming time of mice and decrease the blood lactate and serum urea nitrogen contents. The results provided an important basis for developing the Gl-PS as a novel antioxidant and anti-fatigue compound.

**Key words:** Antioxidant, anti-fatigue, *Ganoderma lucidum*, polysaccharides, forced swimming test

## INTRODUCTION

*Ganoderma lucidum* (Leyss.: Fr.) Karst is a medicinal fungus belonging to the polyporaceae of aphyllophorales (Zhang *et al*., 2003). Its fruiting body is called Lingzhi in China and Reishi in Japan. *Ganoderma lucidum* was first indexed in the Shen Nong's Materia Medica (206 BC-8 AD) as a longevity-promoting and tonic herb of the non-toxic superior class and has been used in Traditional Chinese Medicine (TCM) for >2000 years to prevent and/or treat various human diseases such as chronic bronchitis, hepatitis, hypertension, hypercholesterolemia, chronic fatigue, tumorigenic disease and immunological disorders (Chien *et al*., 2004; Zhu *et al*., 2007). *Ganoderma lucidum* is reported to contain some biologically active components such as polysaccharides and triterpenes (Berovic *et al*., 2003). It has been shown that *Ganoderma lucidum* Polysaccharides (Gl-PS) possess antihyperglycemic, anti-tumor, anti-inflammatory, immunomodulatory and immunotherapeutic activities (Lin and Zhang, 2004; Joseph *et al*., 2011; Li *et al*., 2011). However, few studies have focused on the anti-fatigue activity of Gl-PS.

Moderate exercise is useful for preventing illness and mental stress but excessive exercise itself can be a form of stress and cause fatigue or various types of damage to the organs. Fatigue is the symptom which indicated that the health is about or already subjected to harm (Shang *et al*., 2010; Anand *et al*., 2012). Many reports have indicated that vigorous exercise can cause oxidative stress which leads to an imbalance between Reactive Oxygen Species (ROS) production and antioxidant defense. This imbalance eventually damages biological molecules and key cellular components and processes such as lipid peroxidation, enzyme inactivation and oxidative DNA damage. In recent years, a series of studies showed that exogenous dietary antioxidants can decrease the contribution of exercise-induced oxidative stress and delay physical fatigue. The reason may be that exogenous antioxidants can promote or interact with endogenous antioxidants to form a cooperative network of cellular antioxidants (Powers *et al*., 2004).

The present research investigated the possible antioxidant and anti-fatigue activities of Gl-PS. In this study, the antioxidant activities of Gl-PS were evaluated using various *in vitro* assay systems such as the DPPH and superoxide radical scavenging. *In vivo* anti-fatigue activities of Gl-PS were evaluated using the forced swimming test of animals.

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## MATERIALS AND METHODS

**Chemicals:** DPPH (1,1-Diphenyl-2-Picrylhydrazyl) and BHT (Butylated Hydroxyl Toluene) were purchased from Sigma Chemical Co. (USA). Ascorbic acid was purchased from Zhejiang Wanfeng Chemical Co. (Shaoxing, China). NADH (Nicotinamide Adenine Dinucleotide) and NBT (Nitroblue Tetrazolium) were purchased from Fluka Biochemika AG (Buchs, Switzerland). Blood lactate and Serum Urea Nitrogen (SUN) assay kits were all purchased from Jiancheng Bioengineering Institute (Nanjing, China). All other chemicals and solvents used in this study were of analytical grade and obtained from Hunan Reagent Co. (Changsh, China).

**Materials:** The fruiting bodies of *Ganoderma lucidum* were obtained from a local medicine shop in October 2010 (Changsha, China). The medicinal mushroom was identified by associate professor Li Li (Hunan Normal University, Changsha, China). A voucher specimen was deposited in the Herbarium of Central South University (Changsha, China). Samples were cut into small pieces, dried at 40-50°C for 48 h, ground and passed through 100 mesh screen. The powders were kept in a freezer at -20°C until being used.

**Extraction of *Ganoderma lucidum* polysaccharides:** The method described earlier for the extraction Gl-PS was adapted (Wang *et al*., 2007). Briefly, Gl-PS was extracted by hot water from the *Ganoderma lucidum* fruiting body, followed by ethanol precipitation, dialysis and protein depletion using the Sevag Method. The total yield of Gl-PS was 0.783% (w/w) in terms of the *Ganoderma lucidum* fruiting body. It is a polysaccharide peptide with a molecular weight of 584,900 and has 17 amino acids. The ratio of polysaccharides to peptides is 93.51:6.49%. The polysaccharides consist of rhamnose, xylose, fructose, galactose, mannose and glucose with molar ratios of 0.793:0.964:2.944:0.167:0.384:7.94 and are linked by β-glycosidic linkages.

**Determination of DPPH radical scavenging activity:** DPPH radical scavenging activity was determined according to the method of Chan *et al*. (2007). Briefly, various concentrations of the Gl-PS solutions were prepared. DPPH solution was also prepared by dissolving 6.0 mg of DPPH in 100 mL methanol. Then, 1.0 mL of Gl-PS from each dilution was added into the test tube containing 2.0 mL of DPPH solution. Control was prepared by adding 1.0 mL of methanol to 2.0 mL of DPPH solution. The mixture was shaken vigorously and was left to stand in the dark for 30 min. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm. In the experiment, ascorbic acid and BHT were used as positive controls. The scavenging activity of DPPH radical was calculated using the following equation:

$$\text{Scavenging activity}(\%)=\left(1-\frac{A_{\text{sample at 517 nm}}}{A_{\text{control at 517 nm}}}\right)\times 100$$

**Determination of superoxide anion radical scavenging activity:** The assay was based on the capacity of the sample to inhibit the photochemical reduction of NBT in the Nicotinamide Adenine Dinucleotide-Nitroblue Tetrazolium-Phenazine Methosulfate (NADH-NBT-PMS) System (YouGuo *et al*., 2009; Liang *et al*., 2009). Briefly, various concentrations of the Gl-PS solutions were prepared. About 1.0 mL of NBT solution (156 μM NBT in 100 mM phosphate buffer, pH 7.4), 1.0 mL of NADH solution (468 μM in 100 mM phosphate buffer, pH 7.4) and 0.1 mL of each of the prepared Gl-PS solutions were mixed. After adding 100 μL of PMS solution (60 μM PMS in 100 mM phosphate buffer, pH 7.4) to the mixture, the reaction mixture was incubated at 25°C for 5 min and the absorbance at 560 nm was measured against blank samples. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. In the experiment, ascorbic acid and BHT were used as positive controls. The scavenging activity of hydroxyl radical was calculated using the following equation:

$$\text{Scavenging activity}(\%)=\left(1-\frac{A_{\text{sample at 560 nm}}}{A_{\text{control at 560 nm}}}\right)\times 100$$

**Experimental animals:** Male Kunming strain mice weighing 18-22 g (Grade II, Certificate No. 09-8716) were obtained from Hunan biological supplier (Changsha, China). The animals were maintained at room temperature under alternating 12 h light/dark photoperiod and could access to standard laboratory feed and fresh water *ad libitum*. The approval of this experiment was obtained from the Institutional Animal Ethics Committee of Central South University (Changsha, China).

Mice were trained to accustom themselves to swimming twice (10 min per time) in the 1st week. During the period, the mice which could not learn to swim were screened out. Then the animals were divided into four groups of eight mice each: Control (C) group, Gl-PS low dose treatment (GL) group, Gl-PS Middle dose treatment (GM) group and Gl-PS High dose treatment (GH) group. The control group received Isotonic Saline Solution (ISS, 50 mL $kg^{-1}$ bodyweight) and Gl-PS treatment groups orally obtained 50, 100 and 200 mg $kg^{-1}$ body weight of

Gl-PS in appropriate volumes of physiological saline, respectively. All were administered orally and daily for 4 weeks.

**Determination of anti-fatigue activity:** Anti-fatigue activity of Gl-PS was investigated through forced swimming test of mice. The size of swimming pool was designed as 50×50×40 cm, filled with fresh water at 25±0.5°C. A lead block (5% of body weight) was loaded on the tail roots of the mice. Exhaustion was determined by observing loss of coordinated movements and failure to return to the surface within 10 sec. At this moment, the animals were removed from the swimming pool. They were anaesthetized with sodium pentobarbitate and killed by exsanguination from the carotid artery immediately after exhaustion. Blood were collected for assaying of blood lactate and serum urea nitrogen using commercial kits.

**Statistical analysis:** Results were expressed as means±Standard Deviations (SD). Data were analyzed by using Analysis of Variance (ANOVA) and t-test to the statistical significance ($p<0.05$).

## RESULTS AND DISCUSSION

**DPPH radical scavenging activity of Gl-PS:** The 1, 1-Diphenyl-2-Picrylhydrazyl Radical (DPPH) which possesses an unpaired electron and exhibits a stable violet color in methanol solution (peak absorbance at 517 nm) is commonly used as a reagent for evaluation of the free radical scavenging activity of antioxidants. As shown in Fig. 1 with the increase of concentration, Gl-PS exhibited an excellent DPPH radical scavenging activity which was much higher than those of ascorbic acid and BHT. At 300 mg $mL^{-1}$, the DPPH radical scavenging activity of Gl-PS, ascorbic acid and BHT were 93.1, 81.3 and 52.2%, respectively. The results indicated that Gl-PS has satisfactory scavenging effects on DPPH radical.

**Superoxide anion radical scavenging activity of Gl-PS:** The superoxide radical is known to be very harmful to cellular components as a precursor of more reactive oxygen species. The superoxide radical is known to be produced *in vivo* and can result in the formation of $H_2O_2$ via dismutation reaction. Moreover, the conversion of superoxide and $H_2O_2$ into more reactive species (e.g., the hydroxyl radical) has been thought to be one of the unfavorable effects caused by superoxide radicals (Li and Zhou, 2007). As shown in Fig. 2, Gl-PS exhibited a higher superoxide anion radical scavenging activity than BHT and ascorbic acid. At 300 mg $mL^{-1}$, the DPPH radical scavenging activity of Gl-PS, ascorbic acid and BHT were

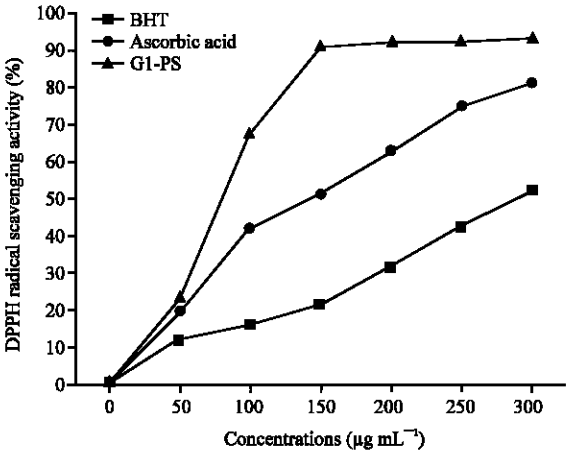


Fig. 1: DPPH radical scavenging activity of Gl-PS

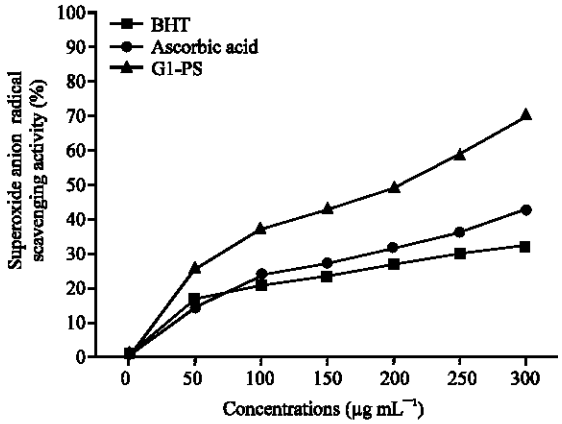


Fig. 2: Superoxide anion radical scavenging activity of Gl-PS

69.7, 42.5 and 31.6%, respectively. The results indicated that Gl-PS has a relatively higher superoxide anion radical scavenging activity.

**Anti-fatigue activity of Gl-PS:** Forced swimming test of mice was selected for evaluation of anti-fatigue activity. The length of the exhaustive swimming time indicates the degree of fatigue (Shang *et al*., 2010). Dawson and Horvath (1970) pointed out that swimming had advantages over other forms of exercise including the treadmill. Training was not required because rodents had a natural swimming ability and they were assumed to be highly motivated to avoid drowning when fatigue was imminent, assuring a high level of performance. As shown in Fig. 3, the exhaustive swimming times of mice in the GL, GM and GH groups were significantly prolonged compared to the C group and the longest times increased by 34.5, 68.3 and 82.4%, respectively. The results indicated that Gl-PS had anti-fatigue activity.

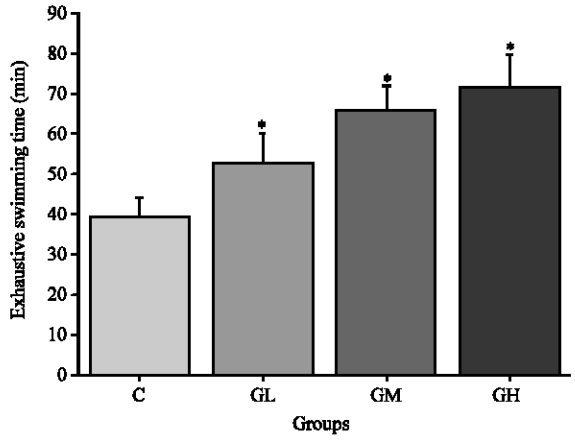


Fig. 3: Effects of Gl-PS on the exhaustive swimming time of mice. *p<0.05 when compared to the Control (C) group

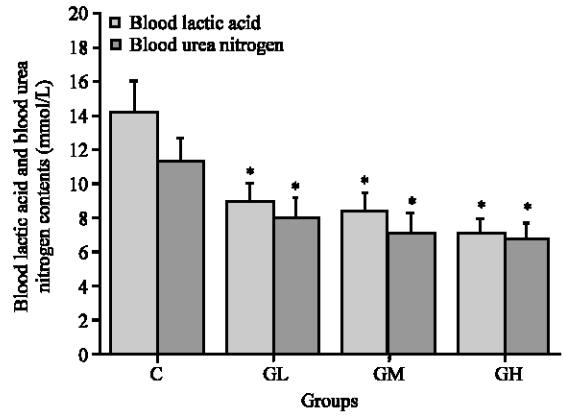


Fig. 4: Effects of Gl-PS on the blood lactate and serum urea nitrogen of mice. *p<0.05 when compared to the Control (C) group

**Biochemical parameters related to fatigue assay:** Blood lactate and Serum Urea Nitrogen (SUN) are important biochemical parameters related to fatigue. 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 (Tang *et al*., 2009). As shown in Fig. 4, the blood lactate content of mice in the GL, GM and GH groups were 9.01±1.03, 8.46±1.06 and 7.11±0.84 mmol $L^{-1}$, respectively which were significantly lower compared to the C group (14.21±1.86 mmol $L^{-1}$). The results indicated that Gl-PS could inhibit the increase of blood lactate and of mice after swimming which was another confirmation that Gl-PS had anti-fatigue activity.

Serum Urea Nitrogen (SUN) is a sensitive index to evaluate the bearing capability when human bodies suffer from a physical load and caused by catabolism of proteins and amino acids. Protein and amino acids have a stronger katabolic metabolism when body cannot obtain enough energy by sugar and fat catabolic metabolism. Therefore, there is a positive correlation between the urea nitrogen *in vivo* and the exercise tolerance (Wei *et al*., 2010). As shown in Fig. 4, the SUN content of mice in the GL, GM and GH groups were 8.03±1.14, 7.29±1.02 and 6.84±0.92 mmol $L^{-1}$, respectively which were significantly lower compared to the C group (11.47±1.23 mmol $L^{-1}$). The results indicated that Gl-PS could reduce catabolic decomposition of protein for energy and ameliorates fatigue.

## CONCLUSION

Gl-PS had *in vitro* antioxidant activities with DPPH and superoxide radical scavenging activity. *In vivo* experimental studies, Gl-PS had anti-fatigue activity which could evidently extend exhaustive swimming time of mice and decrease the blood lactate and serum urea nitrogen contents. The results provided an important basis for developing the Gl-PS as a novel antioxidant and anti-fatigue compound. However, further studies to clarify the detailed mechanisms involved in the anti-fatigue activity of Gl-PS are necessary.

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