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Free radical scavenging of *Ganoderma lucidum* polysaccharides and its effect on antioxidant enzymes and immunity activities in cervical carcinoma rats

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ABSTRACT

Ganoderma lucidum are used as traditional edible and medicinal materials in China. In this study, antioxidant activities of polysaccharides from *G. lucidum* in China were investigated. The influence of *G. lucidum* polysaccharides upon activities of serum antioxidant enzymes and immunity in rats with cervical cancer. The antioxidant activity was measured by $DPPH^-$, O^-, and OH^- free radicals scavenging. Results showed that *G. lucidum* polysaccharides exhibited the higher $DPPH^-$, O^-, and OH^- free radicals scavenging activities. The results still showed that *G. lucidum* polysaccharides could significantly enhance the antioxidant enzyme activities (SOD, CAT and GPx), and reduce levels of IL-1β, IL-6 and TNF-α in rats with cervical cancer.

1. Introduction

Chinese herbal medicines have been widely used for thousands years for the treatment of fractures and joint diseases. *Ganoderma lucidum* is commonly used in traditional Chinese medicine. In the past, the development of herbal anti-osteoporosis formulas was mainly pursued by scientists in Asian countries, including China, Japan and Korea (Hidaka, Okamoto, Yamada, Kon, & Kimura, 1999; Ke et al., 2009; Xu, Dick, Day, Randall, & Prince, 2003). *G. lucidum* (Fr.) Krast (Polyporaceae), a mushroom-like higher fungus, has been a popular folk and an oriental medicine used to treat many diseases, such as hypertension, hypercholesterolemia, leukemia, and gastric cancer (Paterson, 2006). The polysaccharides isolated from *G. lucidum* have the antitumor activities (Li, Fang, & Zhang, 2007; Paterson, 2006; Zhang, Cui, Cheung, & Wang, 2007). Recently, many new highly oxygenated triterpenes have been isolated from the cultured mycelia of *G. lucidum* (Tang, Gu, & Zhong, 2006; Tang, Liu, Zhao, Wei, & Zhong, 2006; Zhou, Yang, & Yang, 2006), and their new biological functions such as inhibiting the proliferation of lung cancer cell line 95-D, anti-HIV-1, and anti-HIV-1 protease have been reported (El-Mekkawy et al., 1998; Lin, Li, Lee, & Kan, 2003; Tang, Liu et al., 2006). In recent years, the submerged fermentation of *G. lucidum* has received great attention for the efficient production of its valuable metabolites, especially *Ganoderma* polysaccharides (Tang & Zhong, 2002) and ganoderic acid (i.e., GA) (Fang & Zhong, 2002; Tang & Zhong, 2003; Zhong & Tang, 2004).

Carcinoma of the cervix is the second most common cancer to affect females worldwide and is the most common cause of cancer-related death in developing countries (Pisani, Parkin, Bray, & Ferlay, 1999). The purpose of this animal study was to examine in vitro free radical scavenging activities and the preventive effects of *G. lucidum* polysaccharides on oxidative injury and immunity activities in rats with cervical cancer. The free radical scavenging activities of *G. lucidum* polysaccharides was measured. The influence of *G. lucidum* polysaccharides upon activities of serum antioxidant enzymes and immunity in rats with cervical cancer were also evaluated.

2. Materials and method

2.1. Extraction of polysaccharides

The fruiting bodies of *G. lucidum* were purchased from a local medicine shop in Yanchen city, china. Sporocarps were cut into small pieces, dried at 40–50 °C for 48 h and powdered. Polysaccharides were isolated by method of Mizuno (2000) and Pillai, Nair, and Janardhanan (2008) and Yin and Dang (2008) with slight modification. The powdered sporocarps 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

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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 (Staub, 1999) several times to remove protein and then dialyzed against deionised water for 48 h at 4 °C. The polysaccharides (crude polysaccharide) were again precipitated with ethanol and the precipitate thus obtained was lyophilized. The crude polysaccharide was dissolved in water and reprecipitated with equal volume of cetyl trimethyl ammonium hydroxide and kept for overnight. The supernatant obtained was precipitated with chilled ethanol. After centrifugation, the precipitate obtained was run through DEAE cellulose column and eluted with deionised water. The precipitate thus obtained was lyophilized to get a light brown powder, (neutral polysaccharide).

2.2. Isolation and purification of Ganoderma lucidum polysaccharides

An aliquot was then applied to an anion-exchange column (5 × 50 cm) of DEAE-Sepharose Fast flow (Pharmacia), and eluted stepwise as two fractions (F1 and F2) (Fig. 1) with 0.1, 0.3, 0.5, 0.7 and 0.9 M NaCl in Tris–HCl buffer (pH 8.5).

2.3. Thin-layer chromatography (TLC)

Thin-layer chromatography (TLC) was performed on a silica gel plate (5 × 20 cm, Silica gel GF254, Qingdao Haiyang Chemical Co.). An aliquot of each sample was spotted onto the silica gel plate with a developing solvent system of chloroform/methanol (10:1, v/v) or pertroleum ether/ethyl acetate (2:1, v/v). The spots were visualised by spraying the plates with spraying solutions of 1% solution of phenylamine- diphenylamine-phosphate in water. Result from thin-layer chromatography (TLC) indicated that F1 and F2 were both composed of mannose (Fig. 2).

2.4. Free radical scavenging of Ganoderma lucidum polysaccharides

2.4.1. Superoxide anion radical-scavenging activity

Superoxide anion radical-scavenging activity was measured by a non-enzymatic method (Nishikimi, Rao, & Yagi, 1972) modified slightly (Kuda, Hishi, & Maekawa, 2006). Sample solution (0.025 ml) was treated with 0.1 ml of 25 mM phosphate buffer (pH 7.2), 2 mM NADH (0.025 ml) and 0.5 mM NBT (0.025 ml),

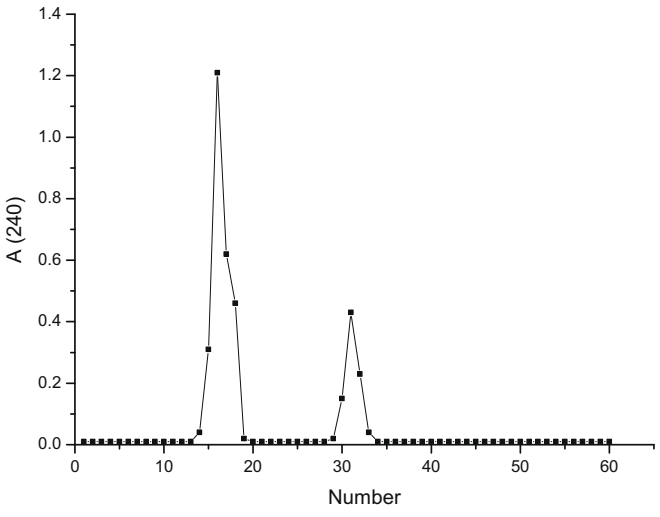

Fig. 1. Isolation and purification of *Ganoderma lucidum* polysaccharides by an anion-exchange column.

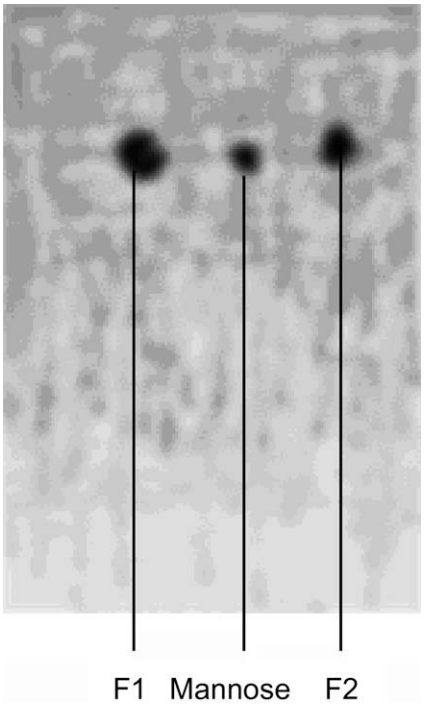

Fig. 2. Thin-layer chromatography.

and absorbance at 560 nm was measured as a blank value. After a 10 min incubation at ambient temperature with 0.025 ml of 0.03 mM PMS, the absorbance was again measured.

2.4.2. Hydroxyl radical-scavenging activity

The hydroxyl radical-scavenging activity was assayed according to the method of Lopes, Schulman, and Hermes-Lima (1999). Briefly, the polysaccharides sample was mixed with a solution containing 5 mM 2-deoxyribose, 100 mM H_2O_2, and 20 mM PBS (pH 7.2). Then, reaction was started by the addition of Fe^{2+} (6 μM final concentration) to this mixture. The reaction was carried out for 15 min at room temperature and stopped by adding 4% phosphoric acid (v/v) and 1% thiobarbituric acid (TBA, w/v, in 50 mM NaOH). After boiling for 15 min at 95° C, sample was cooled to room temperature and the absorbance was read at 532 nm.

2.4.3. Measurement of DPPH free radical-scavenging activity

The DPPH free radical-scavenging activities of the *G. lucidum* polysaccharides extract, fractions, and subfractions derived from *Rhodemela confervoides* were measured using the method in a literature report (Yen & Chen, 1995) as well as our previous publication (Duan, Zhang, Li, & Wang, 2006).

2.5. Animal experiment

2.5.1. Treatment of animals

Thirty-two rats of Wistar strain weighing 170–190 g were purchased from the Central Animal House, Suzhou University. The animals were housed in polypropylene cages and maintained under controlled conditions of 12 h light/12 dark cycle and 50% relative humidity at 25–30 °C. The animals were fed pellet diet and water ad libitum. The study was approved by Institutional Animal Ethics Committee, Yanchen 1th Hospital, Suzhou University and the animals were maintained in accordance with the Guide for the Care and Use of Laboratory Animals. After a period of 1 week, Twenty-four rats were induced cervical cancer according to the reference (Gao, Shi, Di, & Sun, 2008). Then, the animals with cervical cancer