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Modulatory effect of *Ganoderma lucidum* polysaccharides on serum antioxidant enzymes activities in ovarian cancer rats

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ABSTRACT

In the present study, we isolated polysaccharides from *Ganoderma lucidum* and investigated its effect on serum antioxidant enzymes activity in ovarian cancer rats to explore the mechanism underlying the pharmacological anti-cancer activity of th\e polysaccharides. Rats were grouped into the control, model and polysaccharides-treated groups. After experiment ended, serum antioxidant enzymes activity in rats were measured. Results showed that polysaccharides from *G. lucidum* significantly reduced MDA production and increased serum antioxidant enzymes activity. These results suggest that the antioxidant activity of polysaccharides from *G. lucidum* might be benefical towards ovarian cancer therapy.

1. Introduction

Ovarian cancer is the most common cause of cancer death in women (Gupte & Mumper, 2009). Different factors associated with ovarian inflammation may lead to oxidative stress. Reactive oxygen species-mediated tissue injury is a final common pathway for a myriad of disease processes. The body is continuously exposed to free radicals and ROS, from both external sources (sunlight, other forms of radiation, pollution) and generated endogenously. Oxidative stress can cause cancer (Ekambaram, Rajendran, Magesh, & Sakthisekaran, 2008).

Ganoderma lucidum, which belongs to the family of Ganodermataceae of Polyporales, is known as 'Lingzhi' in China and regarded as a panacea, because it is reported to have a broad spectrum of medicinal properties for both health maintenance and treatment of disease. Lingzhi is also called "marvelous herb" or "mushroom of immortality", emphasizing its function in enhancing longevity. Evidence from both in vitro experiments and in vivo animal and human, support Lingzhi extract as a potential anticancer agent (Yuen & Gohel, 2008). It is believed that, at least part of the health benefits of Lingzhi may be attributed to its significant antioxidant action reducing oxidative damage (Mau, Lin, & Chen, 2002; Shi, James, Benzie, & Buswell, 2002; Sun, He, & Xie, 2004).

In the present study, we isolated polysaccharides from *G. lucidum* and investigated its effect on serum antioxidant enzymes activities in ovarian cancer rats.

2. Material 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 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. The polysaccharides content in the precipitate was further determined by phenol–sulphuric acid assay according to Dubois,

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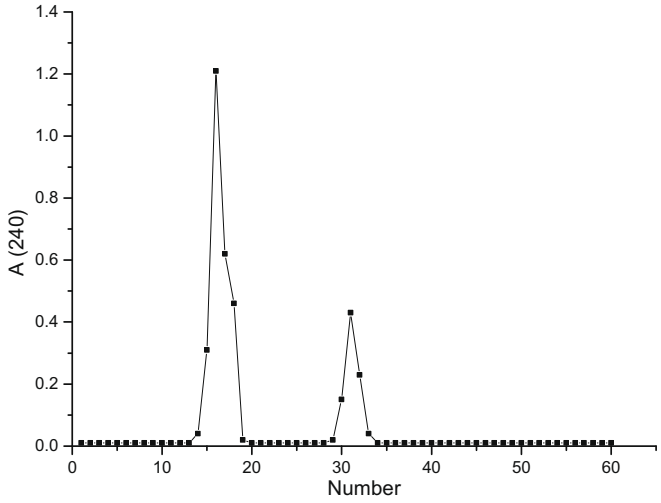

Fig. 1. (Chen et al., 2009). Isolation and purification of *Ganoderma lucidum* polysaccharides by an anion-exchange column.

Gilles, Hamilton, Rebers, and Smith (1956). 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 G. 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 (F 1 and F 2) (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 petroleum 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. Determination of polyphenol content

The polyphenol contents were determined using the Folin–Ciocalteu's reagent as described by Karou, Dicko, Simpore, and Traore, (2005). In brief, 30 μL of either the soluble or hydrolyzable polyphenol extract was mixed with 75 μL of Folin–Ciocalteu's reagent diluted in distilled water (Folin:water, 50:50, v/v). The mixture was allowed to stand for 5 min before adding 75 μL of 20% sodium carbonate solution. After 30 min at room temperature the absorbance was measured at 750 nm using a Beckman 6300 spectrophotometer. The soluble and hydrolyzable polyphenol contents were calculated using a standard curve of gallic acid. The results were expressed as mg of gallic acid equivalents.

2.5. Animal experiment

2.5.1. Treatment of animals

Thirty-two rats of Wistar strain weighing 157–185 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 h 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, Suzhou 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 ten days, Twenty-four rats were induced ovarian cancer according to the reference. Then, the animals with ovarian cancer were divided into three groups of eight rats each and maintained as follows:

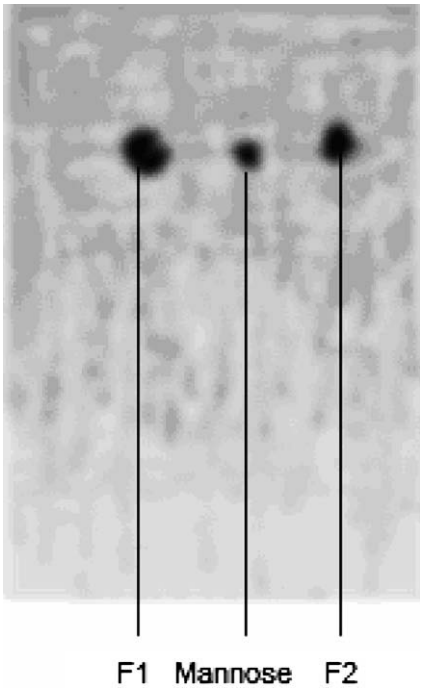

Fig. 2. (Chen et al., 2009). Thin-layer chromatography.

Group I (normal control) received the same volume of physiological saline twice daily.

Group II (model control) received the same volume of physiological saline twice daily.

Group III (low dose of polysaccharides treatment) received polysaccharides (dissolved in 2 ml distilled water) at a dosage of 150 mg/kg body weight twice daily.

Group IV (high dose of polysaccharides treatment) received polysaccharides (dissolved in 2 ml distilled water) at a dosage of 300 mg/kg body weight twice daily.

Food and water were fed ad libitum to all groups. At the end of the experimental period of eight weeks. The rats were sacrificed. Blood was collected in heparinised tubes and plasma was separated. The blood was immediately homogenized in 0.1 M Tris–HCl, pH 7.4. Plasma homogenate was used for various analyses.

2.5.2. Biochemical analysis

2.5.2.1. Superoxide dismutase (SOD) activity. SOD activity was assayed spectrophotometrically as described by (Luchese, Pinton, & Nogueira, 2009). This method is based on the capacity of SOD in inhibiting autoxidation of epinephrine to epinechrome. The color reaction was measured at 480 nm. One unit of enzyme was defined as the amount of enzyme required to inhibit the rate of epinephrine autoxidation by 50% at 26 °C. The enzymatic activity was expressed as Units (U)/mg protein.

2.5.2.2. Catalase (CAT) activity. CAT activity was assayed spectrophotometrically by the method of (Niu, Wu, Yu, & Wang, 2008;

Yu, Wu, & Niu, 2009), which involves monitoring the disappearance of H_2O_2 at 240 nm. One unit of enzyme was defined as the amount of enzyme required for monitoring the disappearance of H_2O_2. The enzymatic activity was expressed as Units (U)/mg protein (1 U decomposes 1 μmol H_2O_2/min at pH 7 at 25 °C).

2.5.2.3. Glutathione peroxidase (GSH-Px) activity. Glutathione peroxidase (GSH-Px) activity was determined according to the method of Chen, Zhong, Zhu, Zeng, and Dai (2009) using cumene hydroperoxide as substrate.

2.5.2.4. TAOC level. The level of TAOC was measured by the method of ferric reducing/antioxidant power assay (Chen, Hu, & Zheng, 2007).

2.5.2.5. TBARS level. TBARS were measured at 532 nm and compared to a standard curve of malondialdehyde (MDA) (Ma, Liu, Yu, Chen, & Zhang, 2009). The amounts of TBARS in serum were expressed as equivalents of MDA.

2.6. Statistical analysis

All data were given as means ± standard deviation (SD). Comparisons between the means of various treatment groups were analyzed using Dunnett's *t*-test followed by analysis of variance (ANOVA). $P < 0.05$ was considered to be significant.

3. Results and discussion

3.1. *Ganoderma lucidum* polysaccharides and polyphenol content

The total phenolic compounds were expressed as *mg gallic acid* equivalent 100 g^{-1}. It could be found that polyphenol content is 152.62 mg gallic acid/100 g polysaccharides. Polysaccharides content is 57.91% (Table 1).

3.2. Effect of G. lucidum polysaccharides on MDA level

The formation of malondialdehyde (MDA)–TBA adducts was used as an index of hydroxyl radical production, by a modified deoxyribose method (Condezo-Hoyos et al., 2009) We have previously demonstrated that *G. lucidum* polysaccharides exerts beneficial antioxidant effects in a rat model of cervical carcinoma, reducing oxidative pressure (Chen et al., in press) As shown in Fig. 3, the MDA level in ovarian cancer model rats was significantly ($P < 0.01$) enhanced when compared to the normal rats. However, the increased MDA level were significantly ($P < 0.01$) reduced in the polysaccharides-treatment groups than in the ovarian cancer model rats.

3.3. Effect of G. lucidum polysaccharides on SOD activity

It has been previously reported that administration of *G. lucidum* polysaccharides prevented GSH depletion and lipid peroxidation, and increases SOD activity in the tissues of rats with the cancer (Shang, Li, Cui, & Hui, 2002. Such antioxidant activity can be explained by the fact that *G. lucidum* polysaccharides stimulated synthesis of enzymes involved in free radical production, creating a powerful redox cycle that allows for continuous cytoprotection against oxidative stress (Chen & Li, 2005). As shown in Fig. 4, SOD activity was significantly ($P < 0.01$) decreased in the ovarian cancer model rats when compared to the normal rats. There was significant difference ($P < 0.01$) in SOD activity between ovarian cancer model rats and polysaccharides treatment rats. It could be found that polysaccharides treatment dose-dependently significantly ($P < 0.01$) increased serum SOD activity in polysaccharides treatment rats.

Table 1
Polysaccharides and polyphenol content.

Polysaccharides content in *Ganoderma lucidum* (%)	Polyphenol content in polysaccharides (mg gallic acid/100 g polysaccharides)
57.91	152.62

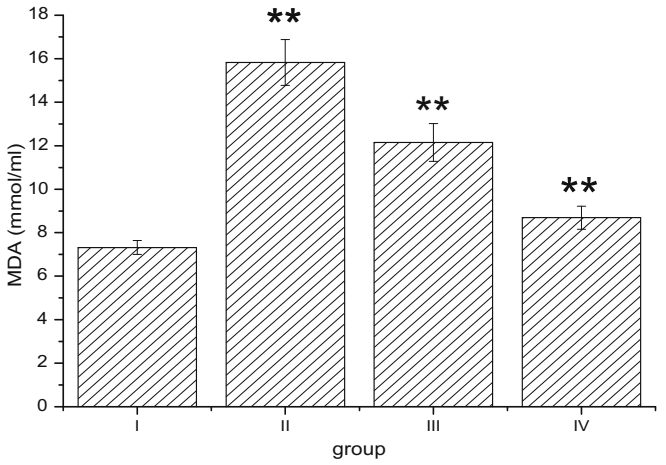

Fig. 3. (Chen et al., 2009). Effect of *Ganoderma lucidum* polysaccharides on MDA level.

3.4. Effect of G. lucidum polysaccharides on CAT activity

It can be found that CAT activity were significantly ($P < 0.01$) decreased in the ovarian cancer model rats compared to normal rats (Fig. 5). By contrast, *G. lucidum* polysaccharides treatment dose-dependently significantly ($P < 0.01$) enhanced CAT activity in the polysaccharides-treatment groups when compared to the ovarian cancer model. The activities of SOD and CAT are known to serve as protective responses to eliminate reactive free radicals (Shi, Sun, He, Guo, & Zhang, 2008). The observation that the levels of SOD and CAT were brought down to their normal levels in the ovarian cancer model rats indicates that the tissues restored to its normal activity by the protective action of *G. lucidum* polysaccharides. Chen and Li (2005) reported that *G. lucidum* water extract possessed effective antioxidant properties and inhibits peroxida-

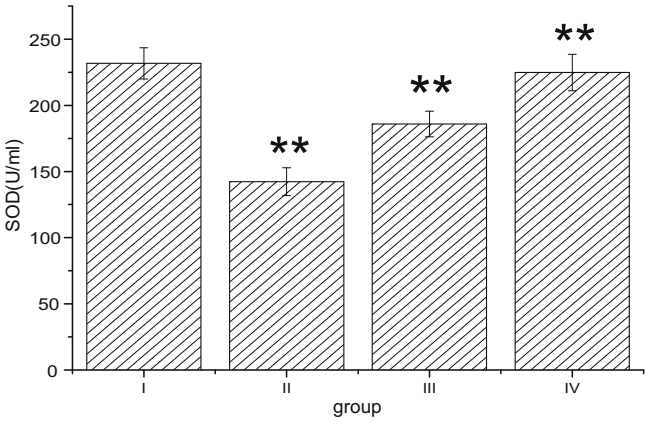

Fig. 4. (Chen et al., 2009). Effect of *Ganoderma lucidum* polysaccharides on SOD activity.

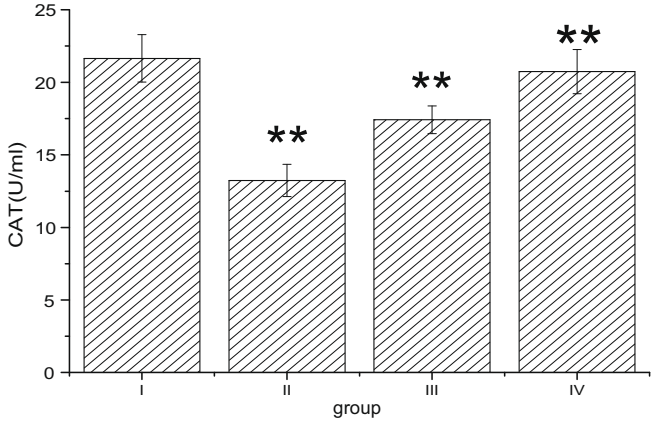

Fig. 5. (Chen et al., 2009). Effect of *Ganoderma lucidum* polysaccharides on CAT activity.

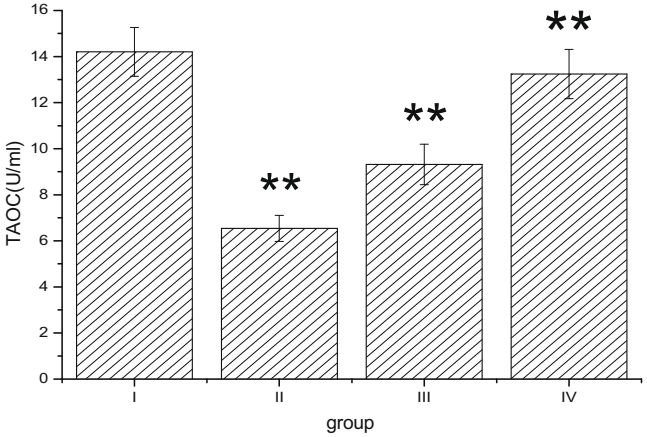

Fig. 7. (Chen et al., 2009). Effect of *Ganoderma lucidum* polysaccharides on TAOC activity.

tion. The results of the present study are in agreement with the reports by. This fact is further substantiated by the decrease in the levels of MDA upon *G. lucidum* polysaccharides administration.

3.5. Effect of G. lucidum polysaccharides on GSH-Px activity

It can be found that GSH-Px activity were significantly ($P < 0.01$) decreased in the ovarian cancer model rats compared to normal rats (Fig. 6). By contrast, *G. lucidum* polysaccharides treatment dose-dependently significantly ($P < 0.01$) enhanced GSH-Px activity in the polysaccharides-treatment groups when compared to the ovarian cancer model. In our study we have observed that MDA, an index of lipid peroxidation, was markedly increased in the ovarian cancer model rats, thus suggesting an increased oxidative stress. Similar data had previously been reported by Lakshmi, Ajith, Jose, and Janardhanan (2006). In addition, total plasma antioxidant capacity was markedly decreased in these animals. The increase of GSH and GSH-Px could be explained by its stimulation to neutralize the increased oxygen free radicals production probably due to the ultra structural damage in the mitochondrial system.

3.6. Effect of G. lucidum polysaccharides on TAOC activity

Total antioxidant capacity (TAOC) reflects the capacity of non-enzymatic antioxidant defense system. Therefore, measure of serum TAOC may give a more precise indication of the relationship between antioxidants and the degenerating process in ovarian cancer model rats (Zhu, Wang, Zhang, Pei, & Fen, 2008). As shown in Fig. 7, TAOC activities significantly decreased ($P < 0.01$) in the ovarian cancer model rats as compared with those in the normal controls. In contrast, the treatment of *G. lucidum* polysaccharides caused a dramatic ($P < 0.01$) increase in serum TAOC activities of polysaccharides-treated rats. A significant difference in activities of TAOC was observed between the ovarian cancer model rats and the ones treated with *G. lucidum* polysaccharides.

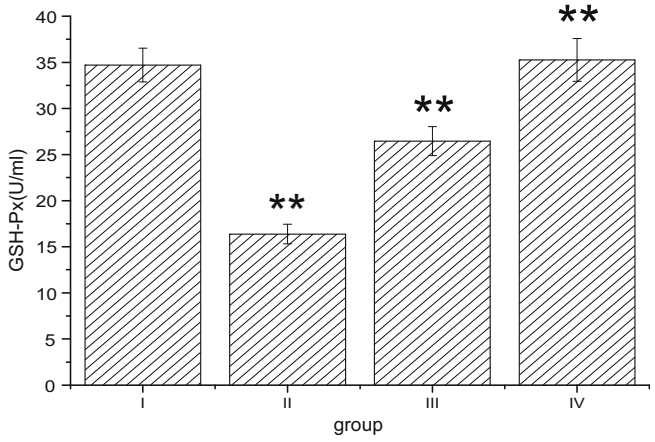

Fig. 6. (Chen et al., 2009). Effect of *Ganoderma lucidum* polysaccharides on GSH-Px activity.

4. Conclusion

For 4000 years *G. lucidum* has been used as a part of Chinese and Japanese medicine especially for the treatment of most of the human ailments. The results of the present study concluded that the doses of *G. lucidum* such as 150 and 300 mg/kg used in this study could improve the antioxidant enzymes activity and reduce oxidative stress such as SOD, CAT, GSH-Px, TAOC activity and MDA level in the serum of ovarian cancer rat which can be partially correlated to its antioxidant activity. However, further studies are needed to investigate its possible protective role against immune system disorders in ovarian cancer rat.

References

Chen, X. P., Chen, Y., Li, S. B., Chen, Y. G., Lan, J. Y., Liu, L. P. (in press). Free radical scavenging of *Ganoderma lucidum* polysaccharides and its effect on antioxidant enzymes and immunity activities in cervical carcinoma rats. *Carbohydrate Polymers*.

Chen, J. R., Hu, T. J., & Zheng, R. L. (2007). Antioxidant activities of Sophora subprosrate polysaccharide in immunosuppressed mice. *International Immunopharmacology, 7*, 547–553.

Chen, W.-Q., & Li, H.-Z. (2005). Effects of *Ganoderma lucidum* polysaccharides on serum lipids and lipoperoxidation in experimental hyper lipidemic rats. *Acta Chinese Medicine and Pharmacology, 33*, 55–56.

Chen, X., Zhong, H. Y., Zhu, H., Zeng, J. H., & Dai, P. (2009). Effect of Curcuma kwangsiensis polysaccharides on blood lipid profiles and oxidative stress in high-fat rats. *International Journal of Biological Macromolecules, 44*, 138–142.

Condezo-Hoyos, L., Abderrahim, F., Victoria Conde, M., Susín, C., Díaz-Gil, J. J., Carmen González, M., et al. (2009). Antioxidant activity of liver growth factor, a bilirubin covalently bound to albumin. *Free Radical Biology and Medicine, 46*, 656–662.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry, 29*, 350–356.

Ekambaram, G., Rajendran, P., Magesh, V., & Sakthisekaran, D. (2008). Naringenin reduces tumor size and weight lost in *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine-induced gastric carcinogenesis in rats. *Nutrition Research, 28*, 106–112.

Gupte, A., & Mumper, R. J. (2009). Elevated copper and oxidative stress in cancer cells as a target for cancer treatment. *Cancer Treatment Reviews, 35*, 32–46.

Karou, D., Dicko, M. H., Simpore, J., & Traore, A. S. (2005). Antioxidant and antibacterial activities of polyphenols from ethnomedicinal plants of Burkina Faso. *African Journal of Biotechnology, 4*, 823–828.

Lakshmi, B., Ajith, T. A., Jose, N., & Janardhanan, K. K. (2006). Antimutagenic activity of methanolic extract of *Ganoderma lucidum* and its effect on hepatic damage caused by benzo[*a*]pyrene. *Journal of Ethnopharmacology, 107*, 297–303.

Luchese, C., Pinton, S., & Nogueira, C. W. (2009). Brain and lungs of rats are differently affected by cigarette smoke exposure: Antioxidant effect of an organoselenium compound. *Pharmacological Research, 59*, 194–201.

Ma, M., Liu, G. H., Yu, Z. H., Chen, G., & Zhang, X. (2009). Effect of the Lycium barbarum polysaccharides administration on blood lipid metabolism and oxidative stress of mice fed high-fat diet in vivo. *Food Chemistry, 113*, 872–877.

Mau, J. L., Lin, H. C., & Chen, C. C. (2002). Antioxidant properties of several medicinal mushrooms. *Journal of Agriculture and Food Chemistry, 50*, 6072–6077.

Mizuno, T. (2000). Development of an antitumor biological response modifier from Phellinus linteus (Berk et curt) Teng (Aphyllophoromycetidae). *International Journal of Medicinal Mushrooms, 2*, 21–33.

Niu, A.-J., Wu, J.-M., Yu, D.-H., & Wang, R. (2008). Protective effect of Lycium barbarum polysaccharides on oxidative damage in skeletal muscle of exhaustive exercise rats. *International Journal of Biological Macromolecules, 42*, 447–449.

Pillai, T. G., Nair, C. K. K., & Janardhanan, K. K. (2008). Polysaccharides isolated from *Ganoderma lucidum* occurring in Southern parts of India, protects radiation induced damages both in vitro and in vivo. *Environmental Toxicology and Pharmacology, 26*, 80–85.

Shang, D.-J., Li, Q.-W., Cui, Q., & Hui, J. (2002). Study on antioxidative and antimutor effect of selenium containing polysaccharide in *Ganoderma lucidum* in mice. *Acta Nutrimenta Sinica, 24*, 249–251.

Shi, Y. I., James, A. E., Benzie, I. F. F., & Buswell, J. A. (2002). Mushroom-derived preparations in the prevention of H_2O_2-induced oxidative damage to cellular DNA. *Teratogenesis Carcinogenesis and Mutagenesis, 22*, 103–111.

Shi, Y. L., Sun, J., He, H., Guo, H., & Zhang, S. (2008). Hepatoprotective effects of *Ganoderma lucidum* peptides against D-galactosamine-induced liver injury in mice. *Journal of Ethnopharmacology, 117*, 415–419.

Staub, A. M. (1999). Removal of protein. Sevag method. *Methods in Carbohydrate Chemistry, 5*, 5–6.

Sun, J., He, H., & Xie, B. J. (2004). Novel antioxidant peptides from fermented mushroom *Ganoderma lucidum*. *Journal of Agricultural and Food Chemistry, 52*, 6646–6652.

Yin, G. H., & Dang, Y. L. (2008). Optimization of extraction technology of the Lycium barbarum polysaccharides by Box–Behnken statistical design. *Carbohydrate Polymers, 74*, 603–610.

Yu, D.-H., Wu, J.-M., & Niu, A.-J. (2009). Health-promoting effect of LBP and healthy Qigong exercise on physiological functions in old subjects. *Carbohydrate Polymers, 75*, 312–316.

Yuen, J. W. M., & Gohel, M. D. I. (2008). The dual roles of Ganoderma antioxidants on urothelial cell DNA under carcinogenic attack. *Journal of Ethnopharmacology, 118*, 324–330.

Zhu, M. Y., Wang, C. J., Zhang, H. S., Pei, X. W., & Fen, J. M. (2008). Protective effect of polysaccharides from *Morinda officinalis* on bone loss in ovariectomized rats. *International Journal of Biological Macromolecules, 43*, 276–278.