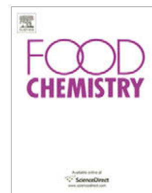
Food Chemistry 115 (2009) 581–584

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



Evaluation of antioxidant and immune activity of *Phellinus ribis* glucan in mice

Chengfu Yuan [a,b], Xiuning Huang [a], Li Cheng [a], Youquan Bu [a], Geli Liu [a], Faping Yi [a], Zhengmei Yang [a], Fangzhou Song [a,*]

[a] *Molecular Medicine & Cancer Research Center, Chongqing Medical University, Chong qing 400016, China*
[b] *Medical School of HuBei Institute for Nationalities, EnShi 445000, China*

ARTICLE INFO

Article history:
Received 2 January 2008
Received in revised form 14 December 2008
Accepted 17 December 2008

Keywords:
Glucan
SOD
Mice
Immune system
Antioxidant

ABSTRACT

The potential protective effects of *Phellinus ribis* glucan (PRG) administration against immune injury due to free radical formation were evaluated in mice. The results showed that glucan administration significantly increased thymus and spleen indices, spleen lymphocyte proliferation and NK cells activity, as well as CD8 T cell numbers, and decreased CD4+/CD8+. In accordance with a previous study, glucan administration significantly enhanced plasma glutathione peroxidase, superoxide dismutases activity and reduced plasma thiobarbituric acid reactive species level. In conclusion, our results indicate that glucan administration improve immune function by its free radicals scavenging activity and reducing oxidative stress in mice.

© 2008 Elsevier Ltd. All rights reserved.

1. Introduction

Increasing evidence highlights that oxidative stress induced cell damage triggers both the physiological process of ageing (Harman, 1993) and many pathological progressions that eventually lead to serious health problems such as Parkinson's and Alzheimer's disease (Giese, Dekker, Barbosa, & da Silva, 2008; Gumustas, Canbaz, & Kaynar, 2007). Free radicals are able to attack numerous biological substance, including lipid membranes, proteins, and DNA, and exert some detrimental effects, including lipid peroxidation of cell membranes, alteration of lipid-protein interactions, enzyme inactivation, and DNA breakage (Halliwell & Gutteridge, 1998).

In recent years, numerous research has been performed to find out fundamental physicochemical properties of glucan (Yu, Wu, & Niu, 2009; Zhang et al., 2005). β-glucans are glucose polymers found in the cell walls of yeast, fungi and cereal plants. Currently, β-glucans are accepted to be one of the most powerful immune response modifiers (Brown & Gordon, 2003). More than 2000 papers describing the biological activities of glucans exist. Glucan and its derivatives have shown various functional properties and made them possible to be used in many fields including food, agriculture, and medicine (Ajithkumar, Andersson, Siika-aho, Tenkanen, & Åman, 2006; Surenjav, Zhang, Xu, Zhang, & Zeng, 2006). Recent advances have demonstrated the health benefits of glucan including lowering of blood cholesterol, lowering of high blood pressure, protective effects against infections and free radicals, controlling arthritis and enhancing antitumour properties (Hida, Miura, Adachi, & Ohno, 2005). Several mechanisms were proposed for the protective effect of β-glucan, one of them is related to antioxidant capacity of the molecule (Şener, Toklu, & Cetinel, 2007).

Phellinus ribis is a kind of fungi belonging to *Phellinus genus*, *Hymenochaetaceae* family. The acidic polysaccharide isolated from Phellinus linteus enhanced cell-mediated immunity, and the protein-bound polysaccharide had a direct anti-tumour effect (Ren, Liu, Zhu, Yang, & Fu, 2006) and (Li et al., 2004). Extracts of *Phellinus conchatus*, *Phellinus baumii* and *Phellinus rimosus* showed cytotoxic, free radical scavenging and antihepatotoxic activity, respectively (Ajith & Janardhanan, 2002; Ren et al., 2006).

In the present study, the immunopharmacological activities of glucan from the fruiting bodies of *P. ribis* were investigated by using parameters related with T cells, B cells and macrophages. The relationship of immune function improvement and antioxidant activity with glucan treatment is also discussed.

2. Materials and methods

2.1. Materials

Acetylsalicylic acid was purchased from Sigma (Sigma–Aldrich, Zwijndrecht, The Netherlands). Recombinant mouse IL-3 and stem

Abbreviations: DMSO, dimethyl sulphoxide; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; NK, natural killer cells; PRG, *Phellinus ribis* glucan; TBARS, thiobarbituric acid reactive species.

* Corresponding author. Tel./fax: +86 23 68485073.
E-mail address: yuan_chenfu@yahoo.cn (F. Song).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.12.055

cell factor were obtained from Peprotech (Sanbio, Uden, The Netherlands). Fluorescently labelled monoclonal anti-TLR2 (6C2), anti-TLR4 (MTS510) and isotype control antibodies were purchased from eBiosciences, Inc. (San Diego, CA, USA). All other chemicals were purchased from Sigma (St. Louis, MO, USA).

P. ribis was collected from mountain area in lanzhou city, Gansu Province, China, and identified by Professor F.J. Tao, School of Traditional Chinese Medicine, Lanzhou University. Voucher specimens (No. 2007005721) were preserved in the Natural Product Research Institute.

2.2. Extraction of polysaccharide

Glucan was extracted from *P. ribis* according to Liu and Wang (2007). Dried crushed fruiting bodies (2.0 kg) of *P. ribis* were extracted with distilled water (8 L) at 90 °C every 6 h for four times, filtered through gauze and centrifuged at 3000*g* for 15 min to remove water-insoluble materials. The aqueous extract was concentrated at 55 °C under vacuum and treated with four volumes of ethanol at 4 °C overnight. The resulting precipitate was collected, dissolved in water and deproteinated by a combination of trypsin enzymolysis and Sevag method (Staub, 1965). The crude polysaccharide fraction (56.7 g) was obtained through precipitation with four volumes of ethanol and desiccation under vacuum.

2.3. Animals and experimental design

Fifty kunming mice (20–23 g), aged about 60 days, were obtained from Experimental Animal Center of Chongqing Medical University (ChongQing, China). All animal procedures were approved by Animal Care and Use Committee of ChongQing Medical University (ChongQing city, China). The mice, which were housed in accordance with institutional animal care policies, had free access to water and standard laboratory chow. Animals were randomly divided into five groups: normal controls, lingzhi polysaccharides treatment group (positive control), glucan treatment groups (I, II, III) ($n = 10$ in each group). Lingzhi polysaccharides group was fed with polysaccharides in a dose of 300 mg/kg body weight per day treated to mice by gavage for 30 days. Glucan groups (I, II, III) were, respectively, fed with glucan in three different doses: 100, 200, and 300 mg/kg body weight per day treated to mice by gavage for 30 days. Normal control was given only the vehicle by oral gavage for 30 days.

2.4. The relative thymus and spleen weight

After 30 days of oral administration, mice were weighed and sacrificed by cervical dislocation. Spleen and thymus were excised from the animal and weighed immediately. Thymus index was expressed as the thymus weight relative to body weight. Spleen index was expressed as the thymus weight relative to body weight.

2.5. Lymphocyte proliferation assay

Spleens were aseptically removed from sacrificed mice with scissors and forceps in 0.1 M cold phosphate-buffered saline (PBS), gently homogenised with a loose Teflon pestle and passed through a sterilized mesh (200 mesh) to obtain single cell suspensions according to Yuan, Song, Li, Li, and Dai (2006). Erythrocytes in the cell mixture were washed by the hypo-osmostic haemolysis rapidly. Finally, the cells were suspended to a final density of 5×10^6 cells/ml in RPMI-1640 medium supplemented with 10% newborn bovine serum (Invitrogen Corporation, Carlsbad, CA, USA). Spleen cells (100 μl/well) were seeded into a 96-well plate in the presence of ConA (8 μg/ml) and cultured at 37 °C in 5% CO_2 atmosphere. After incubation for 72 h, 10 μl MTT (5 mg/ml) was added to each well and the plate was incubated for another 4.5 h. The plate was then centrifuged at 200*g* for 15 min and the supernatants were discarded. A total of 100 μl dimethyl sulphoxide (DMSO) were added to each well and shaken until all crystals dissolved. The absorbance at 570 nm was detected on a XD711 microplate reader (Shanghai Xunda Medical Instrument Co., Ltd, Shanghai, China).

2.6. Assay of natural killer (NK) cells activity

All procedures were conducted under aseptic conditions by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Choi, Kim, Kim, & Hwang, 2005). Spleen cells prepared as described above (in a concentration of 2×10^6 cells/ml) together with YAC-1 cells (in a concentration of 1×10^5 cells/ml) were incubated in the wells of a flat bottom 96 well sterile cell culture plate (Effector: Target ratio of 50:1) with a total volume of 100 μl in each well (ET). The plates were then incubated for 20 h at 37 °C in 5% CO_2 atmosphere. One hundred millilitre of supernatants were collected from each well and 10 μl MTT (5 mg/ml) were added to each well and after another 4 h of incubation, the plate was subjected to MTT cellular assay. Three kinds of control measurements were performed: target cells control, blank control and effector cells control. The optical density (OD) of each well was measured using an XD711 microplate reader (Shanghai Xunda Medical Instrument Co., Ltd, Shanghai, China). The percentage of NK cell activity was determined by the formula: % NK cell activity = 1 − [(optical density value of test samples − optical density value of effector cells control)/optical density value of target cells control] × 100.

2.7. Spleen lymphocyte T-cell subgroup

In antibody blocking experiments, 0.1 ml spleen lymphocyte cells suspension (6×10^8 cells/L) were incubated with 10 μl of anti-CD4 or anti-CD8 antibodies, obtained from mice for 60 min at 4 °C. The cells were then washed twice in (phosphate-buffered saline) PBS, and resuspended in 1% paraformaldehyde (PFA) in PBS on completion of the staining procedure. CD4 and CD8 T cell numbers were measured by means of flow cytometry.

2.8. Antioxidant assay

Blood samples (3 ml) from each mouse were obtained prior to the morning meal on the last day of the experiment and then centrifuged at 3000*g* for 10 min at 4 °C to obtain serum. Serum was stored at −30 °C for analysing levels of TBARS (thiobarbituic acid reactive substances), the activity of SOD and and GSH-Px by spectrophotometric methods. The TBARS levels in serum were expressed as malondialdehyde (MDA) equivalents (Shahidi & Ho, 2007; Thijssen et al., 2007).

The SOD activity was analysed according to the method of Ilhan et al. (2005). The activity of SOD in serum was expressed in units per 1 ml of serum. The activity of GSH-Px in serum was measured according to the method of Kayali and Tarhan (2006) and the result was expressed in units per 1 ml of serum.

2.9. Statistical analysis

Unless otherwise indicated, the results were expressed as the means ±SD of data obtained from triplicate experiments ($n = 10$). Statistical analysis was performed by a paired *t*-test. P values < 0.05 were considered statistically significant.

3. Results

3.1. Effects of glucan on thymus and spleen indexes in mice

As shown in Table 1, a statistically significant increase ($P < 0.05$, $P < 0.01$) in the thymus and spleen indices was noted in the positive control (lingzhi polysaccharides treatment) group compared with normal control group. The thymus and spleen indices (100× spleen or thymus weight/body weight) in the glucan treatment groups (II, III) significantly ($P < 0.01$) increased compared with the normal control group, but there was no significant difference at the low dose of glucan level (I) ($p > 0.05$). Moreover, the glucan treatment exhibited a stronger effect on the thymus index and the spleen index compared with the lingzhi polysaccharides-treated mice at a dose of 300 mg/kg body weight.

3.2. Effects of glucan on cellular immunity in mice

Spleen lymphocyte proliferation and NK cells activity were examined. The results showed that glucan treatment significantly ($P < 0.05$, $P < 0.01$) increased spleen lymphocyte proliferation and NK cells activity in mice (I, II, III) in a dose-depended manner, and that lingzhi polysaccharides treatment also displayed strong effect on spleen lymphocyte proliferation and NK cells activity in the positive control (lingzhi polysaccharides treated) mice in comparison with the normal control ($P < 0.05$, $P < 0.01$). In addition, it could be found that the glucan treatment exhibited a stronger effect on spleen lymphocyte proliferation and NK cells activity compared with the positive control (lingzhi polysaccharides treated mice) at the dose of 300 mg/kg body weight (Table 2).

3.3. Analysis of effect of glucan administration on CD4 and CD8 T cell numbers

As shown in Table 3, no significant difference ($P > 0.05$) was found regarding CD4 T cell counts between groups (normal control group, positive control, glucan treatment groups) (Table 3). The number of CD8 T cells in normal control group was significantly ($P < 0.01$) lower than that found in the positive control (lingzhi polysaccharides-treated) group. Compared with the normal control group, glucan treatment dose-dependently enhanced the number of CD8 T cells in glucan treatment groups (I, II, III), but only displayed statistically significant ($P < 0.05$, $P < 0.01$) difference at middle and high dose levels (II, III). As shown in Table 3, CD4+/CD8+ in glucan treatment groups (II, III) were found to significantly increase in a dose-dependent manner compared to the normal control group. The CD4+/CD8+ in the positive control (lingzhi polysaccharise-treated) group were lower than the glucan treatment group (III) at the dose of 300 mg/kg body weight.

Table 1
Effects of glucan on thymus and spleen indices in mice.

Group	Thymus index	Spleen index
Control	0.27 ± 0.03	0.41 ± 0.03
Lingzhi polysaccharides	0.38 ± 0.04[b]	0.49 ± 0.02[a]
Glucan(I)	0.29 ± 0.02	0.45 ± 0.04
Glucan(II)	0.34 ± 0.02[b]	0.52 ± 0.06[b]
Glucan(III)	0.39 ± 0.04[b]	0.58 ± 0.03[b]

Thymus and spleen indices was expressed as 100× spleen or thymus weight/body weight.
[a] $p < 0.05$, compared to normal control.
[b] $p < 0.01$, compared to normal control.

Table 2
Effects of glucan on spleen lymphocyte proliferation and NK cells activity in mice.

Group	Lymphocyte proliferation (A_{570})	NK activity (%)
Control	0.45 ± 0.03	61.56 ± 4.27
Lingzhi polysaccharides	0.79 ± 0.05[b]	70.41 ± 5.75[a]
Glucan(I)	0.54 ± 0.02[a]	67.36 ± 4.42[a]
Glucan(II)	0.63 ± 0.06[b]	74.40 ± 5.04[b]
Glucan(III)	0.83 ± 0.07[b]	78.48 ± 6.05[b]

[a] $p < 0.05$, compared to normal control.
[b] $p < 0.01$, compared to normal control.

Table 3
Comparison of mice spleen Lymphocyte subgroup between groups.

Group	$CD4^+$	$CD8^+$	$CD4^+/CD8^+$
Control	19.33±1.53	10.46 ± 0.93	2.01 ± 0.42
Lingzhi polysaccharides	18.65 ± 1.79	14.78 ± 1.67[b]	1.42 ± 0.09[b]
Glucan(I)	20.21 ± 2.06	11.22 ± 1.78	1.97 ± 0.71
Glucan(II)	20.81 ± 2.35	13.25 ± 1.64[a]	1.64 ± 0.38[b]
Glucan(III)	19.26 ± 2.94	15.95 ± 1.06[b]	1.28 ± 0.17[b]

[a] $p < 0.05$, compared to normal control.
[b] $p < 0.01$, compared to normal control.

Table 4
Effect of glucan on plasma TBARS level, SOD and GSH-Px activities.

Group	TBARS (nmol/ml)	SOD (NU/ml)	GSH-Px (U/ml)
Control	7.35 ± 0.89	5.47 ± 0.43	2.57 ± 0.08
lingzhi polysaccharides	5.43 ± 0.64[b]	7.59 ± 0.37[b]	3.56 ± 0.21[b]
Glucan(I)	6.53 ± 0.52[a]	6.02 ± 0.54[a]	2.78 ± 0.18
Glucan(II)	5.92 ± 0.44[b]	7.16 ± 0.66[b]	3.68 ± 0.27[b]
Glucan(III)	4.37 ± 0.35[b]	8.25 ± 0.95[b]	4.21 ± 0.33[b]

[a] $p < 0.05$, compared to normal control.
[b] $p < 0.01$, compared to normal control.

3.4. Effect of glucan on plasma antioxidant status

Table 4 shows the GSH-Px and SOD activities as well as TBARS levels. The level of TBARS among blood samplings in glucan treatment group (I, II, III) were found to significantly ($P < 0.05$, $P < 0.01$) decrease compared to the normal control group. A significant increase of SOD activity ($P < 0.05$, $P < 0.01$) was noticed in the glucan treatment groups (I, II, III) in comparison with the normal control group (Table 4). The GSH-Px activities in the glucan treatment groups (II, III) significantly ($P < 0.01$) increased compared with the normal control group, but there was no significant difference at the low dose of glucan (I) ($p > 0.05$). Moreover, the results still displayed that glucan showed stronger antioxidant activities than lingzhi polysaccharides at a dose of 300 mg/kg body weight (Table 4).

4. Discussion

Glucan administration markedly enhanced the thymus and spleen indices in mice and cellular immune response. It was demonstrated that the administration of glucan elicited significantly higher NK cells activity, spleen lymphocyte proliferation, and expression of IFN-γ- producing T cells in mice. This may indicate that glucan improve immune function activity of the mice. This phenomenon was also observed by Zhao, Kan, Li, and Chen (2005), who reported an enhanced cellular immune response to the same extent.

Urao, Fujimoto, Lane, Seo, and Miyano (1999) suggested that the glucan administration allowed the beneficial microorganisms to quickly reproduce in the animal intestine, e.g. bifidobacterium.

These beneficial microorganisms can synthesise vitamin, amino acid, etc, and to stimulate immunoglobulin activity and improve immune function. In addition, there are some reports on the immune–antioxidant activity relationship of glucan (Toklu, Özer Şehirli, Velioğlu-Öğünç, Çetinel, & Şener, 2006; Şener, Ekşioğlu-Demiralp, Çetiner, Ercan, & Yeğen, 2006; Şener et al., 2007; Matheson & Caldwell, 2008). Many studies have revealed that antioxidants with higher antioxidant activity in certain *in vitro* systems could also improve the immune function. Cumulative free radicals induce oxidative damage on mice lymphocytes (Sklavos, Tse, & Piganelli, 2008). In this study, administration of glucan significantly ($P < 0.05$; $P < 0.01$) enhanced the spleen lymphocyte proliferation and NK cells activities. A major component of the antioxidant system in mammalian cells consists of three enzymes, namely, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px). These enzymes work in concert to detoxify ROS such as $O_2^{\cdot-}$ and H_2O_2 in cells, which is further converted to H_2O with the help of GPx and CAT. Higher levels of the antioxidant enzymes have been correlated with decreased susceptibility to cell damage. Şener et al. (2006) and Şener et al. (2007) found that the administration of glucan was advantageous to promote the activities of SOD and GSH-Px in mouse. In our study, administration of glucan obviously raised the activities of antioxidant enzymes (SOD and GSH-Px) and reduced the level of TBARS. In our opinion, immunomodulatory effect of glucan is closely associated with its strong antioxidant activity. The protective effect of glucan on immune function may be due to the improvement in the reduced activities of antioxidant enzymes.

5. Conclusions

The results of immunity and antioxidant activities *in vivo* of PRG show that PRG could dose-dependently significantly enhance thymus and spleen indices, spleen lymphocyte proliferation and NK cells activity, SOD and GSH-Px activities, and reduce CD4+/CD8+, TBARS level, respectively. This work demonstrated that PRG might have some ideal immune-modulatory and antioxidant characteristics, and inhibitory effect on lipid peroxidation and may be utilised as a good natural immune-modulatory and antioxidant agent in the food and medicinal fields.

References

Ajith, T. A., & Janardhanan, K. K. (2002). Antioxidant and antihepatotoxic activities of *Phellinus rimosus* (Berk) Pilat. *Journal of Ethnopharmacology, 81*, 387–391.

Ajithkumar, A., Andersson, R., Siika-aho, M., Tenkanen, M., & Åman, P. (2006). Isolation of cellotriosyl blocks from barley β-glucan with endo-1, 4-β-glucanase from *Trichoderma reesei*. *Carbohydrate Polymers, 64*, 233–238.

Brown, G. D., & Gordon, S. (2003). Fungal β-glucans and mammalian immunity. *Immunity, 19*, 311–315.

Choi, E. M., Kim, A. J., Kim, Y. O., & Hwang, J. K. (2005). Immunomodulating activity of arabinogalactan and fucoidan in vitro. *Journal of Medicinal Food, 8*, 446–453.

Giese, E. C., Dekker, R. F. H., Barbosa, A. M., & da Silva, R. (2008). Triple helix conformation of botryosphaeran, a (1→3;1→6)-β-d-glucan produced by *Botryosphaeria rhodina* MAMB-05. *Carbohydrate Polymers, 74*, 953–956.

Gumustas, K., Canbaz, B., & Kaynar, M. Y. (2007). Relationship between DNA damage and total antioxidant capacity in patients with glioblastoma multiforme. *Clinical Oncology, 19*, 177–181.

Halliwell, B., & Gutteridge, J. M. C. (1998). *Free radicals in biology and medicine*. New York: Oxford University Press. pp. 105–107.

Harman, D. (1993). Free radical involvement in aging: pathophysiology and therapeutic implications. *Drugs and Aging, 3*, 60–80.

Hida, S., Miura, N. N., Adachi, Y., & Ohno, N. (2005). Effect of *Candida albicans* cell wall glucan as adjuvant for induction of autoimmune arthritis in mice. *Journal of Autoimmunity, 25*, 93–101.

Ilhan, A., Aladag Arif, M., Kocer, A., Boluk, A., Gurel, A., & Armutcu, F. (2005). Erdosteine ameliorates PTZ-induced oxidative stress in mice *seizure* model. *Brain Research Bulletin, 65*, 495–499.

Kayali, H. A., & Tarhan, L. (2006). The relationship between the levels of total sialic acid, lipid peroxidation and superoxide dismutase, catalase, glutathione peroxidase, ascorbate antioxidant in urea supplemented medium by *Fusarium* species. *Enzyme and Microbial Technology, 39*, 697–702.

Li, G., Kim, D. H., Kim, T. D., Park, B. J., Park, H. D., Park, J. I., et al. (2004). Protein-bound polysaccharide from *Phellinus linteus* induces G2/M phase arrest and apoptosis in SW480 human colon cancer cells. *Cancer Letters, 216*, 175–181.

Liu, Y. H., & Wang, F. S. (2007). Structural characterization of an active polysaccharide from *Phellinus ribis*. *Carbohydrate Polymers, 70*, 386–392.

Matheson, N. K., & Caldwell, R. A. (2008). Modeling of α(1–4) chain arrangements in α(1–4)(1–6) glucans: The action and outcome of β-amylase and Pseudomonas stutzeri amylase on an α(1–4)(1–6) glucan model. *Carbohydrate Polymers, 72*, 625–637.

Ren, G., Liu, X. Y., Zhu, H. K., Yang, S. Z., & Fu, C. X. (2006). Evaluation of cytotoxic activities of some medicinal polypore fungi from China. *Fitoterapia, 77*, 408–410.

Şener, G., Ekşioğlu-Demiralp, E., Çetiner, M., Ercan, F., & Yeğen, B. Ç. (2006). Β-glucan ameliorates methotrexate-induced oxidative organ injury via its antioxidant and immunomodulatory effects. *European Journal of Pharmacology, 542*, 170–178.

Şener, G., Toklu, H. Z., & Cetinel, S. (2007). Β-Glucan protects against chronic nicotine-induced oxidative damage in rat kidney and bladder. *Environmental Toxicology and Pharmacology, 23*, 25–32.

Shahidi, F., & Ho, C.-T. (2007). *Antioxidant Measurement and Applications. ACS Symposium Series, 956*. Washington, DC: American Chemical Society.

Sklavos, M. M., Tse, H. T., & Piganelli, J. D. (2008). Redox modulation inhibits CD8 T cell effector function. *Free Radical Biology and Medicine, 45*, 1477–1486.

Staub, A. M. (1965). Removal of protein—Sevag method. *Methods in Carbohydrate Chemistry, 5*, 5–6.

Surenjav, U., Zhang, L. N., Xu, X. J., Zhang, X. F., & Zeng, F. B. (2006). Effects of molecular structure on antitumor activities of (1→3)-β-d-glucans from different *Lentinus Edodes*. *Carbohydrate Polymers, 63*, 97–104.

Thijssen, S., Cuypers, A., Maringwa, J., Smeets, K., Horemans, N., Lambrichts, I., & Kerkhove, E. V. (2007). Low cadmium exposure triggers a biphasic oxidative stress response in mice kidneys. *Toxicology, 236*, 29–41.

Toklu, H. Z., Özer Şehirli, A., Velioğlu-Öğünç, A., Çetinel, Ş., & Şener, G. (2006). Acetaminophen-induced toxicity is prevented by β-D-glucan treatment in mice. *European Journal of Pharmacology, 543*, 133–140.

Urao, M., Fujimoto, T., Lane, G. J., Seo, G., & Miyano, T. (1999). Does probiotics administration decrease serum endotoxin levels in infants? *Journal of Pediatric Surgery, 34*, 273–276.

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.

Yuan, H. M., Song, J. M., Li, X. G., Li, N., & Dai, J. C. (2006). Immunomodulation and antitumor activity of κ-carrageenan oligosaccharides. *Cancer Letters, 243*, 228–234.

Zhang, L. N., Chen, L., Xu, X. J., Lin, Y. L., Cheung, P. C. K., & Kennedy, J. F. (2005). Comparison on chain stiffness of a water-insoluble (1→3)-α-D-glucan isolated from *Poria cocos* mycelia and its sulfated derivative. *Carbohydrate Polymers, 59*, 257–263.

Zhao, G. H., Kan, J. Q., Li, Z. X., & Chen, Z. D. (2005). Characterization and immunostimulatory activity of an (1→6)-*a*-d-glucan from the root of *Ipomoea batatas*. *International Immunopharmacology, 5*, 1436–1445.