

The Immunostimulatory and Anti-tumor Activities of Polysaccharide from *Agaricus bisporus* (brown)

Yanqing Zhang, Guijie Ma, Leilei Fang, Lijuan Wang, Junbo Xie*

College of Biotechnology and Food Science, Tianjin University of Commerce, Tianjin, China

*Corresponding author: xjbo@tjcu.edu.cn

Received March 11, 2014; Revised March 31, 2014; Accepted April 09, 2014

Abstract *Agaricus bisporus* is one of the most consumed culinary mushrooms in the world. The white type of *Agaricus bisporus* has been demonstrated with various bioactivities beneficial to human health. However, little was known about its brown type. In this study, the anti-cancer and immunoregulatory potential of *Agaricus bisporus* (brown) polysaccharide were investigated *in vitro* and *in vivo*. *In vitro*, the polysaccharide had a significant antiproliferative effect on Hela cells (cervical cancer cells), especially at the high concentration of 200 µg/ml. The spleen index, thymus index and carbon clearance ability of mice were remarkably increased by the polysaccharide treatment, indicating the polysaccharide had a promising benefit for immunodulation *in vivo*. Furthermore, the polysaccharide increased the proliferation of RAW264.7 cells with 19.1% at 50µg /ml and 34.5% at 100µg /ml, which demonstrated the immunodulation mechanism was in close conjunction with macrophages. In conclusion, these results indicated that *Agaricus bisporus* (brown) polysaccharide possessed strong immunostimulatory and anti-tumor bioactivity *in vivo* and *in vitro*. Therefore, it could be explored as a novel natural antitumor agent with immunomodulatory activity.

Keywords: *Agaricus bisporus* (brown), polysaccharide, immunostimulatory, anti-tumor, Hela cells, RAW264.7 cells

Cite This Article: Yanqing Zhang, Guijie Ma, Leilei Fang, Lijuan Wang, and Junbo Xie, "The Immunostimulatory and Anti-tumor Activities of Polysaccharide from *Agaricus bisporus* (brown)." *Journal of Food and Nutrition Research*, vol. 2, no. 3 (2014): 122-126. doi: 10.12691/jfnr-2-3-5.

1. Introduction

Agaricus bisporus is one of the most commonly and widely consumed culinary mushrooms in European and America [1]. It has been revealed that *Agaricus bisporus* contains various abundant nutritional substances, such as proteins [2], polysaccharides [3], fatty acids [4], vitamins [5], and phenols [6].

Agaricus bisporus is divided into two types (white and brown) based on the color of its pileus. Although it is reported that the two types show similar macronutrients composition (contents of total sugars and total tocopherols), there is also considerable difference in chemical composition between them [2,7]. In the recent years, much research has demonstrated that *Agaricus bisporus* (white) exhibit various biological effects, such as antioxidant [8], immunomodulating [9,10], cancer preventive [11,12], aromatase inhibiting [13,14] and anti-hepatic steatosis [15]. By contrast, few studies were performed to investigate the effect of *Agaricus bisporus* (brown) [16]. It is still unclear whether *Agaricus bisporus* (brown) could exert the similar effects as the white type, especially in immunomodulation and anti-cancer.

It is well known that polysaccharide is the main bioactive component of *Agaricus bisporus* [17,18]. For example, (1→6)-β-D-glucans from the mushroom could

present an obvious immunostimulatory activity, which contribute to the antitumor effects [19]. In the present study, the anticancer potential of *Agaricus bisporus* (brown) polysaccharide extract was investigated with determining its antiproliferative effect on *Hela* Cells. In addition, the immunomodulatory activity was also investigated *in vitro* (murine RAW264.7 macrophages) and *in vivo* (mice).

2. Materials and Methods

2.1. Materials

Agaricus bisporus (brown) was from Jinsannong agricultural technology development company Ltd. (Tianjin, China), and the voucher specimen was deposited in the Department of Pharmaceutical Engineering, Tianjin University of Commerce.

AB-8 macroporous adsorptive resin was from Nanda resin technology company Ltd. (Tianjin, China). D201 ion exchange resin was from Baoen adsorptive material company Ltd. (Cangzhou, China). Glucose was obtained from Troody technology company Ltd. (Shanghai, China). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and Dimethyl sulfoxide (DMSO) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). DMEM culture medium, fetal bovine serum (FBS),

Trypsin-EDTA, and Penicillin-Streptomycin were purchased from Gibco/Invitrogen (Grand Island, NY, USA). All other reagents were all of analytical grade.

2.2. Methods

2.2.1. Extraction and Purification of the Polysaccharide

The mushroom were freeze-dried and milled into 60 mesh particle size, then weighed (200g) and extracted with 5000ml distilled water (100°C) for 2h. The extraction solution was collected and centrifuged at 3000rpm for 15 min. The obtained supernatant was adjusted to be 10mg/ml (calculated as Glucose assayed with phenol-sulfuric acid method [20]). The columns (2.6 cm × 100 cm) of AB-8 and D201 were used for decoloration and deproteination of the crude polysaccharides solution respectively. After dialysis (molecular weight cut-off 10000 Da), the solution were freeze-dried, and the polysaccharide was obtained. The results of UV analysis showed that there was no absorbance at 280nm, which indicated that the proteins were removed totally with the procedure.

2.2.2. Hela Cells Growth Inhibition Assay

Hela cells (cervical cancer cells) were obtained from Shanghai Institute of Cell Biology (Shanghai, China). The cells were cultured in DMEM medium containing 10%FBS and 100 IU/ml Penicillin-Streptomycin at 37°C in a 5% CO₂ atmosphere. The experiment was performed at the logarithmic growth phase of the cells.

The inhibition effect of *Agaricus bisporus* (brown) polysaccharides on the growth of Hela cells was assayed by MTT method [21]. Hela cells were collected and suspended at the density of 5×10⁴ cells/ml in DMEM medium. After the cell suspension was transferred into a 96-well plate (100μl/well) and inoculated for 24 h, different concentrations of *Agaricus bisporus* (brown) polysaccharides (0, 50,100, 200 μg/ml in fresh growth medium) was used to replace the original medium separately. Then, the cells were incubated for 24 h at 37°C. After the medium was removed, 100μl of MTT solution (0.5 mg/ml, in culture medium) was added to each well. The cells were incubated for another 4 h. Then, the supernatant was removed carefully and 150μl DMSO was added to dissolve formazan crystals. The OD value was assayed at 490 nm by SpectraMax® M3 microplate reader (MDC, U.S.A). All the experiments were performed 5 times and the inhibitory rate was calculated as follows:

$$\text{Inhibition rate (\%)} = (1 - \text{OD}_1 / \text{OD}_0) \times 100\% (\text{OD}_1 : \text{sample}; \text{OD}_0 : \text{control}).$$

2.2.3. RAW264.7 Cells Proliferation Assay

RAW264.7 cells (mouse macrophage-like cell line) were also obtained from Shanghai Institute of Cell Biology (Shanghai, China), and were cultured as the reported method [22]. The effect of *Agaricus bisporus* (brown) polysaccharides on the proliferation of RAW264.7 cells was assayed with the same MTT method described in “2.2.2”. Different concentrations of *Agaricus bisporus* (brown) polysaccharides (0, 25, 50, 100μg/ml) were used evaluate the effect. All the experiments were

performed 5 times and the relative proliferation rate was calculated as follows: relative proliferation rate (%) = (OD₁/OD₀) × 100% (OD₁: sample; OD₀: control).

2.2.4. Animals and Experimental Design

Kunming mice (4–5 weeks, 11-15g) were obtained from the Experimental Animal Center of Tianjin (Certificate No. SCXK 2010-0002). All the experiments were approved by the university animal care and use committee. The animal handling procedures were pursuant to the national guidelines for laboratory animals.

The spleen and thymus indexes were assayed in accordance with previous reported method [23]. 40 Kunming mice were randomly divided into four groups (10 in each group), i.e. control group (20 ml·kg⁻¹·d⁻¹ physiological saline), low dosage group (96mg·kg⁻¹·d⁻¹ polysaccharides), middle dosage group (120mg·kg⁻¹·d⁻¹ polysaccharides) and high dosage group (150mg·kg⁻¹·d⁻¹ polysaccharides). According to the grouping protocol, the animals were treated (intra-gastric administration) with the physiological saline and polysaccharides for successive 8 days, based on the body weights respectively. One hour after the last administration, the experimental mice were weighed and sacrificed by cervical dislocation. Then, the spleens and thymus were excised and weighted, respectively. Thymus index was calculated as the thymus weight / body weight (balanced before and after experiment), and spleen index was the spleen weight / body weight (balanced before and after experiment).

The carbon clearance test *in vivo* was performed just as the described procedure [24]. Just as in the experiment of assaying spleen index and thymus index, 24 mice were divided into 4 groups (6 in each group), including control group, low dosage group, middle dosage group and high dosage group. The animals were also treated (intra-gastric administration) with the physiological saline and polysaccharides for successive 7 days. On the 8th day, the mice were weighed and injected with carbon ink suspension (10mL·kg⁻¹) via the tail vein. After 30s (t₁) and 6 min(t₂), 50μl blood samples were drawn from the retroorbital vein respectively, and mixed with 4 ml sodium carbonate solution (0.1%, w/v). The absorbance was measured at 675 nm. The carbon clearance ability was expressed as:

$$K = (\text{Lg}A_1 - \text{Lg}A_2) \cdot (t_2 - t_1)$$

A₁ and A₂ are the absorbance at 30s and 6 min, respectively [23].

2.2.5. Statistical Analysis

All data are expressed as mean ± S.E.M. The analysis was performed with one-way analysis of variance (ANOVA) and Student's t-test. Statistical significance was considered when p value is less than 0.05. All statistical analyses were performed by SPSS 13.0 software.

3. Results and Discussion

3.1. The Inhibition Effect on Growth of Hela Cells

Hela cells (cervical cancer cells) were incubated 24 h in the presence of *Agaricus bisporus* (brown) polysaccharide

at concentration of 0, 50, 100 and 200 $\mu\text{g}/\text{ml}$. With the MTT method, the inhibition effect of the polysaccharide on growth of Hela cells was investigated. Just as shown in Figure 1, it was demonstrated that the polysaccharide exerted dose dependent toxicity activity on Hela cells, and 200 $\mu\text{g}/\text{ml}$ polysaccharide decreased the cell viability significantly ($p < 0.01$) with the inhibitory rate of 13.8%. Thus, *Agaricus bisporus* (brown) polysaccharide was presumably capable of inhibiting the growth of Hela cells.

Much research has demonstrated that *Agaricus bisporus* (white) presents specific preventive effects on various cancers, including sarcoma [9], prostate cancer [12], breast cancer [13] and liver hepatocellular carcinoma [17]. However, there are few previous reports on anti-cancer effect of *Agaricus bisporus* (brown). In the present study, the result verified the promising antiproliferative effect of *Agaricus bisporus* (brown) polysaccharide on Hela cells (cervical cancer cells) for the first time.

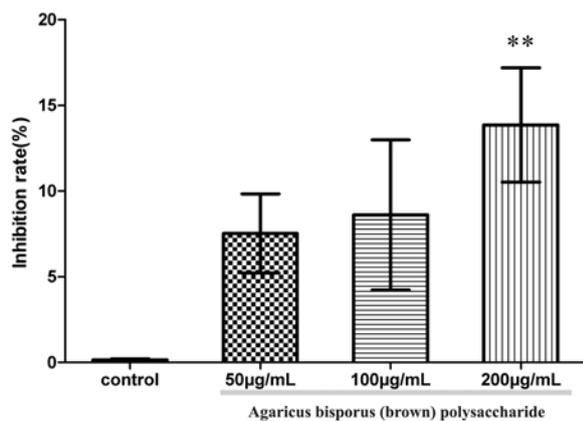


Figure 1. Inhibition of Hela cells growth by different concentrations of *Agaricus bisporus* (brown) polysaccharide. The values are presented as mean \pm S.E.M. ($n=5$). * * Significant difference at $p < 0.01$ levels compared with control group

3.2. Effects of *Agaricus bisporus* (brown) Polysaccharide on Spleen Index, Thymus Index and Carbon Clearance Ability in Vivo

To evaluate the immunoregulatory activity of *Agaricus bisporus* (brown) polysaccharide *in vivo*, the Kunming mice were administered with the polysaccharide for 8 days.

Then, the spleens and thymuses were excised and weighted to determine the spleen and thymus indexes. As shown in Figure 2, *Agaricus bisporus* (brown) polysaccharide exhibited a dose dependent improving effect on the thymus index and spleen index, especially at the high dose of 150 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$ ($p < 0.05$) with significance. Moreover, the polysaccharide also exerted significant increase in carbon clearance ability (phagocytic index) at 120 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$ ($p < 0.05$). However, this effect was not dose dependent (Figure 3). The results indicated that *Agaricus bisporus* (brown) polysaccharide had a predictable benefit for immunodulation in Kunming mice.

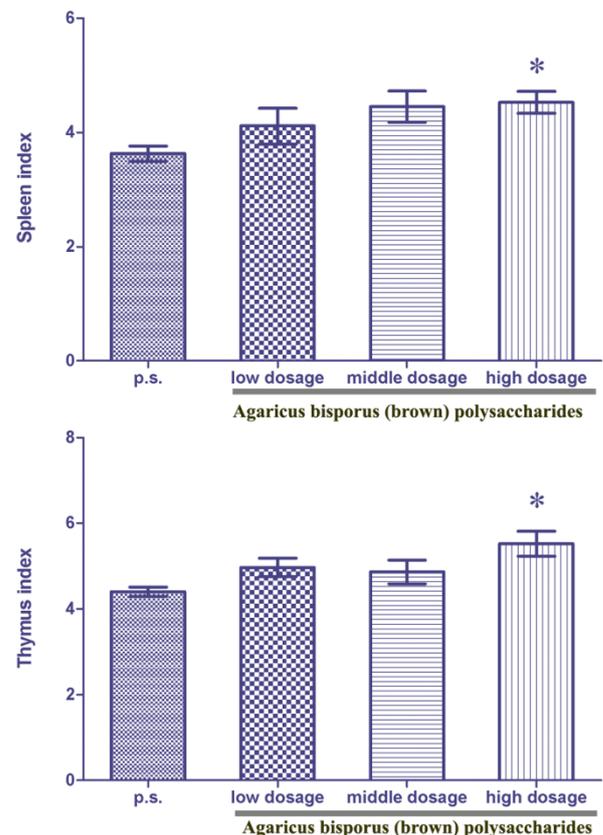


Figure 2. The effects of *Agaricus bisporus* (brown) polysaccharide on mice spleen and thymus indexes. The values are presented as mean \pm S.E.M. ($n = 10$). A: spleen index; B: thymus index. * Significant difference at $p < 0.05$ levels compared with P.S. group

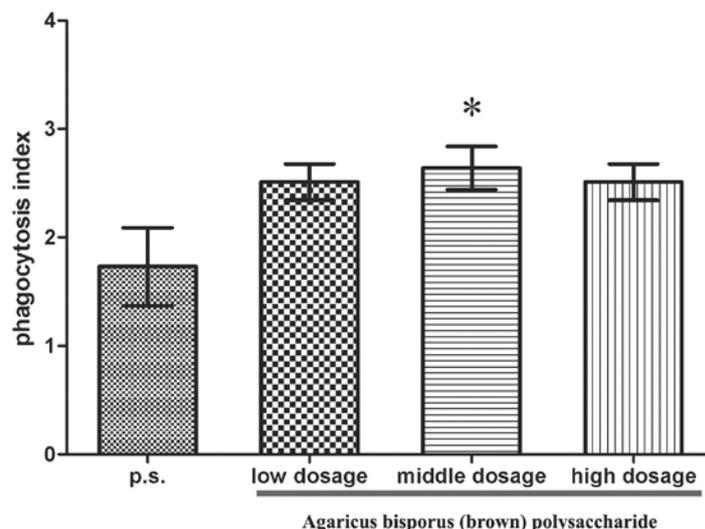


Figure 3. The effects of *Agaricus bisporus* (brown) polysaccharide on mice carbon clearance ability. The values are presented as mean \pm S.E.M. ($n=6$). * Significant difference at $p < 0.05$ levels compared with P.S. group

The anti-cancer effect is traditionally postulated to be not only pertinent to the direct effect on cancer cells, but the ability of modulating immune system function [10,17,25]. Spleen and thymus are the main immune organs, and their indexes can reflect the condition of immunologic function [26,27]. The main function of thymus is producing T-lymphocytes (T cells), which are critical cells of the adaptive immune system and responsible for cellular immunity. Spleen possesses plentiful macrophages and lymphocytes, in which B cells are in a large proportion and closely associated with the humoral immunity. The present study manifested that *Agaricus bisporus* (brown) polysaccharide could increase the spleen and thymus indexes significantly, indicating that the polysaccharide played a crucial role in immunostimulatory activity.

Phagocytosis is a vital immune defence process in which leukocytes engulf malignant cells, pathogenic microorganisms, tissue debris and inorganic particles [24]. In this study, the carbon clearance test was performed to evaluate the phagocytosis effect of *Agaricus bisporus* (brown) polysaccharide in mice. As the carbon particles came into the systemic circulation, it would be cleared up by reticuloendothelial system involving phagocytes. The results showed that *Agaricus bisporus* (brown) polysaccharide increased the phagocytic index remarkably at $120\text{mg}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$ in mice. This effect is owing to the phagocytosis mechanism in conjunction with macrophages.

3.3. The Effects of *Agaricus bisporus* (brown) Polysaccharide on RAW264.7 Cells Proliferation

To further elucidate immunological effect of *Agaricus bisporus* (brown) polysaccharide, the effects of *Agaricus bisporus* (brown) polysaccharide on mouse macrophage RAW264.7 cells proliferation was investigated. The polysaccharide exerted a dose dependent improving effect on the proliferation of RAW264.7 cells (Figure 4). Compared with the control group, the polysaccharide increased the proliferation with 19.1% at $50\mu\text{g}/\text{ml}$ ($p<0.05$), and 34.5% at $100\mu\text{g}/\text{ml}$ ($p<0.01$). The results indicated that *Agaricus bisporus* (brown) polysaccharide played a promising role in improving RAW264.7 cells proliferation.

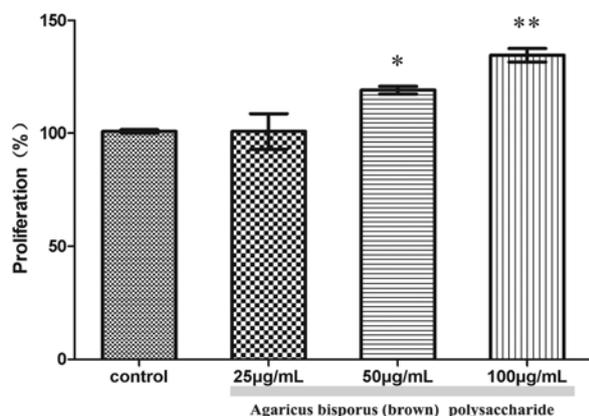


Figure 4. The effects of *Agaricus bisporus* (brown) polysaccharide on RAW264.7 cells proliferation. The values are presented as mean \pm S.E.M. ($n=5$). *Significant difference at $p<0.05$ levels compared with control group; ** Significant difference at $p<0.01$ levels compared with control group

The mouse macrophage-like cell line RAW264.7, is the most commonly used mouse macrophage cell line in medical research. For further investigating the effect of *Agaricus bisporus* (brown) polysaccharide on macrophages, the role of different dose polysaccharide in RAW264.7 cell proliferation was evaluated with MTT method. It was indicated that *Agaricus bisporus* (brown) polysaccharide exerted significant ameliorating effect on RAW264.7 cell proliferation, which was congruent with the result of carbon clearance *in vivo*. The previous report demonstrated that the *Agaricus bisporus* (brown) could decrease the NO production with IC50 values of less $0.1\text{mg}/\text{ml}$, but not affect RAW264.7 cell viability in the tested range [22]. This indicated that administration of low dose *Agaricus bisporus* (brown) polysaccharide has little effect on the RAW264.7 cell viability. It has been considered that the medical mushrooms exerted their positive impacts on human health through regulating the immune system. The anti-cancer effect of *Agaricus bisporus* (brown) might also attribute to its considerable immunomodulating bioactivity.

Acknowledgement

This research was supported by Important Project Foundation of Tianjin China (No. 12ZCZDNC01400).

Statement of Competing Interests

The authors have no competing interests.

List of Abbreviations

ANOVA, one-way analysis of variance; DMSO, dimethylsulfoxide; FBS, fetal bovine serum; MTT, 3-(4, 5-dimethylthiazol-2-yl)-2, 5- diphenyltetrazolium bromide; S.E.M., standard error of measurement

References

- Beelman RB., Royse DJ. and Chikhimma N., "Bioactive components in Button mushroom *Agaricus bisporus* (J. Lge) Imbach (Agaricomycetidae) of nutritional, medicinal, and biological importance," *International Journal of Medicinal Mushrooms*, 5. 321-327. 2003.
- Reis FS., Barros L., Martins A. and Ferreira IC., "Chemical composition and nutritional value of the most widely appreciated cultivated mushrooms: an inter-species comparative study," *Food and Chemical Toxicology*, 50. 191-197. 2012.
- Smiderle FR., Ruthes AC., van Arkel J., Chanput W., Iacomini M., Wichers HJ., and van Griensven LJ., "Polysaccharides from *Agaricus bisporus* and *Agaricus brasiliensis* show similarities in their structures and their immunomodulatory effects on human monocytic THP-1 cells," *BMC Complementary & Alternative Medicine*, 11. 58. 2011.
- Öztürk M., Duru ME., Kivrak S., Mercan-Doğan N., Türkoglu A. and Özler MA., "In vitro antioxidant, anticholinesterase and antimicrobial activity studies on three *Agaricus* species with fatty acid compositions and iron contents: a comparative study on the three most edible mushrooms," *Food and Chemical Toxicology*, 49. 1353-1360. 2011.
- Çağlarımak N., "Chemical composition and nutrition value of dried cultivated culinary-medicinal mushrooms from Turkey," *International Journal of Medicinal Mushrooms*, 13. 351-356. 2011.
- Piljac-Zegarac J., Samec D., Piljac A., Mesić A. and Tkalcic Z., "Antioxidant properties of extracts of wild medicinal mushroom

- species from Croatia.” *International Journal of Medicinal Mushrooms*, 13. 257-263. 2011.
- [7] Mattila P., Könkö K., Eurola M., Pihlava JM., Astola J., Vahteristo L., Hietaniemi V., Kumpulainen J., Valtonen M. and Piironen V., “Contents of vitamins, mineral elements, and some phenolic compounds in cultivated mushrooms,” *Journal of Agriculture and Food Chemistry*, 49. 2343-2348. 2001.
- [8] Liu J., Jia L., Kan J. and Jin CH., “In vitro and in vivo antioxidant activity of ethanolic extract of white button mushroom (*Agaricus bisporus*),” *Food and Chemical Toxicology*, 51. 310-316. 2013.
- [9] Jeong SC., Koyyalamudi SR., Jeong YT., Song CH. and Pang G., “Macrophage immunomodulating and antitumor activities of polysaccharides isolated from *Agaricus bisporus* white button mushrooms,” *Journal of Medical Food*, 15. 58-65. 2012.
- [10] Ren Z., Guo Z., Meydani SN. and Wu D., “White button mushroom enhances maturation of bone marrow-derived dendritic cells and their antigen presenting function in mice,” *Journal of Nutrition*, 138. 544-550. 2008.
- [11] Xu T., Beelman RB. and Lambert JD., “The cancer preventive effects of edible mushrooms,” *Anticancer Agents in Medicinal Chemistry*, 12. 1255-1263. 2012.
- [12] Adams LS., Phung S., Wu X., Ki L. and Chen S., “White button mushroom (*Agaricus bisporus*) exhibits antiproliferative and proapoptotic properties and inhibits prostate tumor growth in athymic mice,” *Nutrition and Cancer*, 60. 744-756. 2008.
- [13] Grube BJ., Eng ET., Kao YC., Kwon A. and Chen S., “White button mushroom phytochemicals inhibit aromatase activity and breast cancer cell proliferation,” *Journal of Nutrition*, 131. 3288-3293. 2001.
- [14] Chen S., Oh SR., Phung S., Hur G., Ye JJ., Kwok SL., Shrode GE., Belury M., Adams LS. and Williams D., “Anti-aromatase activity of phytochemicals in white button mushrooms (*Agaricus bisporus*),” *Cancer Research*, 66. 12026-12034. 2006.
- [15] Kanaya N., Kubo M., Liu Z., Chu P., Wang C., Yuan YC. and Chen S., “Protective effects of white button mushroom (*Agaricus bisporus*) against hepatic steatosis in ovariectomized mice as a model of postmenopausal women,” *Plos One*, 6. e26654. 2011.
- [16] Kohno K., Miyake M., Sano O., Tanaka-Kataoka M., Yamamoto S., Koya-Miyata S., Arai N., Fujii M., Watanabe H., Ushio S., Iwaki K. and Fukuda S., “Anti-inflammatory and immunomodulatory properties of 2-amino-3H-phenoxazin-3-one,” *Biological & Pharmaceutical Bulletin*, 31. 1938-1945. 2008.
- [17] Pires Ado R., Ruthes AC., Cadena SM., Acco A., Gorin PA. and Iacomini M., “Cytotoxic effect of *Agaricus bisporus* and *Lactarius rufus* β -D-glucans on HepG2 cells,” *International Journal of Biological Macromolecules*, 58. 95-103. 2013.
- [18] Ruthes AC., Rattmann YD., Malquevicz-Paiva SM., Carbonero ER., Córdova MM., Baggio CH., Santos AR., Gorin PA. and Iacomini M., “*Agaricus bisporus* fucogalactan: structural characterization and pharmacological approaches,” *Carbohydrate Polymers*, 92. 184-191. 2013.
- [19] Smiderle FR., Alquini G., Tadra-Sfeir MZ., Iacomini M., Wichers HJ., Van Griensven LJ., “*Agaricus bisporus* and *Agaricus brasiliensis* (1 \rightarrow 6)- β -D-glucans show immunostimulatory activity on human THP-1 derived macrophages,” *Carbohydrate Polymers*, 94. 91-99. 2013.
- [20] Dubois M., Gilles KA., Hamilton JK., Rebers PA. and Smith F., “Colorimetric method for determination of sugars and related substances,” *Analytical Chemistry*, 28. 350-356. 1956.
- [21] Liu L., Jia J., Zeng G., Zhao Y., Qi X., He C., Guo W., Fan D., Han G. and Li Z., “Studies on immunoregulatory and anti-tumor activities of a polysaccharide from *Salvia miltiorrhiza* Bunge,” *Carbohydrate Polymers*, 92. 479-483. 2013.
- [22] Gunawardena D., Bennett L., Shanmugam K., King K., Williams R., Zabarar D., Head R., Ooi L., Gyengesi E. and Münch G., “Anti-inflammatory effects of five commercially available mushroom species determined in lipopolysaccharide and interferon- γ activated murine macrophages,” *Food Chemistry*, 148. 92-96. 2014.
- [23] Gao HY., Li GY., Huang J., Han Y., Sun FZ., Du XW., An LJ., Wang HY. and Wang JH., “Protective effects of Zhuyeqing liquor on the immune function of normal and immunosuppressed mice in vivo,” *BMC Complementary & Alternative Medicine*, 13. 252. 2013.
- [24] Singh S., Yadav CPS. and Noolvi M N., “Immunomodulatory activity of butanol fraction of *Gentiana olivieri* Griseb. on Balb/C mice,” *Asian Pacific journal of tropical biomedicine*, 2. 433-437. 2012.
- [25] Borchers AT., Stern JS., Hackman RM., Keen CL. and Gershwin ME., “Mushrooms, tumors and immunity,” *Proceedings of the Society for Experimental Biology and Medicine*, 221. 281-293. 1999.
- [26] Xu WB., Xu LH., Lu HS., Ou-Yang DY., Shi HJ., Di JF. and He XH., “The immunosuppressive effect of gossypol in mice is mediated by inhibition of lymphocyte proliferation and by induction of cell apoptosis,” *Acta Pharmacological Sinica*, 30. 597-604. 2009.
- [27] Götling YY., Fritsch Fredin M., Kumawat AK., Strid H., Willén R., Rangel I., Bland PW. and Hörnquist EH., “Interplay between T(h)1 and T(h)17 effector T-cell pathways in the pathogenesis of spontaneous colitis and colon cancer in the *Gai2*-deficient mouse,” *International Immunology*, 25. 35-44. 2013.