Differences in antioxidant activities of outdoor- and indoor-cultivated *Agaricus brasiliensis*, and protective effects against carbon tetrachloride-induced acute hepatic injury in mice

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**Abstract**

**Background:** *Agaricus brasiliensis* (*A. brasiliensis*) is a medicinal mushroom that exerts various pharmacological actions. We previously demonstrated that different cultivation conditions altered the activity of the polyphenol-related enzymes from this mushroom. However, the influence of cultivation conditions on the antioxidant activity of the fruiting bodies remains unclear. Therefore, in this study we compared the antioxidative effects of fruiting bodies of *A. brasiliensis* cultivated outdoors and indoors. In addition, we assessed whether different cultivation methods affected the hepatoprotective effects against CCl₄-induced liver injury.

**Methods:** We assessed the antioxidative effects of mushrooms cultivated in open-air or indoors using the DPPH radical-scavenging assay. Furthermore, we prepared experimental feeds containing outdoor- or indoor-cultivated *A. brasiliensis*. Acute liver injury was induced by CCl₄ injection in mice that consumed feed containing outdoor- or indoor-cultivated *A. brasiliensis*. The hepatoprotective effects of these mushrooms were then evaluated by monitoring the reduction in the circulating levels of alanine aminotransferase, aspartate aminotransferase, and lactate dehydrogenase. The significance of the differences between the means was assessed using Student’s t-test. Finally, histopathological analysis of liver was performed.

**Results:** In the DPPH assay, the antioxidant activity of outdoor-cultivated *A. brasiliensis* was higher than that of indoor-cultivated mushroom. Moreover, in the mouse model of CCl₄-induced hepatitis, the oral administration of outdoor-cultivated *A. brasiliensis* reduced liver damage significantly, but indoor-cultivated mushrooms failed to inhibit hepatitis. The hepatoprotective effects of outdoor-cultivated *A. brasiliensis* were observed even when ingestion commenced only 1 day before CCl₄ injection, and these effects were not affected by excessive heat treatment.

**Conclusions:** Outdoor cultivation significantly enhanced the antioxidative activity of *A. brasiliensis* fruiting bodies. In addition, outdoor-cultivated *A. brasiliensis* was more effective at protecting against CCl₄-induced liver injury in mice than mushrooms grown in a greenhouse.

**Keywords:** *Agaricus blazei*, Cultivation condition, Sunlight, Hepatitis, Antioxidative activity
Agaricus brasiliensis (A. brasiliensis) S. Wasser et al. (also known as A. blazei Murrill sensu Heinemann, A. ruftegulis and A. subrubescens) [1-3] is a medicinal mushroom used worldwide as a biological response modifier [4,5]. To date, many reports demonstrated beneficial effects of A. brasiliensis on human health. For example, natural killer cell activity was upregulated after the intake of the fruiting body of A. brasiliensis without any adverse effects [6]. The immunoenhancing effects of this mushroom are principally exerted by abundant β-glucans [7,8], and several innate immune receptors such as Toll-like receptor (TLR) 2 [9], TLR4 [10], and dectin-1 [11] also contribute to the augmentation of host immune systems stimulated by A. brasiliensis. Evidence of pharmaceutical activity has improved the reliability of A. brasiliensis as a functional food.

In addition to immunoenhancing activities, we identified additional beneficial effects of A. brasiliensis, such as the protection against concanavalin A- or LPS-induced murine liver injury, after oral administration in water extracts [6]. The oral administration of extracts of A. brasiliensis fruiting bodies or mycelium also exhibited hepatoprotective effects in a model of CCl4-induced hepatitis [12,13]. Although the underlying mechanism(s) behind its hepatoprotective effects remain unclear, the antioxidative activity of A. brasiliensis could contribute to its activity because inhibiting the generation of free radicals is important for protecting against CCl4-induced hepatic injury [13,14]. Therefore, the antioxidative properties of this mushroom may be important for the treatment of free radical-induced hepatitis.

Hepatitis remains an incurable disease. In particular, viral hepatitis is highly infectious, and so is a global health issue. Many patients who are unable receive effective pharmaceutical agents choose to use traditional medicines such as A. brasiliensis due to its hepatoprotective activities. Today, many commercial products based on this mushroom are available, and patients can select one of these products. However, we previously demonstrated that different A. brasiliensis cultivation conditions (such as open air or indoors) affected its protein and chemical components significantly. In particular, outdoor cultivation strongly upregulated the activity of peroxidase and laccase [15]. These enzymes contribute to the degradation or composition of phenolic compounds, and, thus, cultivation conditions may influence their antioxidative properties and related biological activities. Therefore, to assess the optimum use of A. brasiliensis, we investigated the effect of cultivation conditions on its antioxidative activity.

In the present study, we assessed the influence of cultivation conditions on the antioxidant activities of three kinds of A. brasiliensis mushroom and compared the hepatoprotective effects of outdoor- and indoor-cultivated A. brasiliensis on CCl4-induced murine hepatic injury.

Methods

Animals and materials

Male ICR mice (4 weeks of age) were purchased from Japan SLC (Shizuoka, Japan). The mice were housed in a specific pathogen-free environment, and were used at 5–6 weeks of age. All experiments were performed in accordance with the guidelines for laboratory animal experiments provided by the Tokyo University of Pharmacy and Life Sciences, and the Committee for Laboratory Animal Experiments at Tokyo University of Pharmacy and Life Sciences (P13-47) approved each experimental protocol. 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was purchased from Tokyo Chemical Industry Co., Ltd, Tokyo, Japan. 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was purchased from Calbiochem Inc., CA, USA. Carbon tetrachloride was purchased from Wako Pure Chemical Co. (Osaka, Japan), and olive oil was obtained from Nikko Pharmaceutical Co., Ltd. (Gifu, Japan).

A. brasiliensis fruiting bodies

A. brasiliensis strain KA21, deposited at National Institute of Technology and Evaluation, Japan with deposition number: FERM P-17695 was cultivated outdoors with sunlight or indoors avoiding sunshine, using the same compost. The fruiting bodies were harvested in Brazil, washed, and dried using hot air at 60°C or lower (Toei Shinyaku Co., Ltd., Tokyo, Japan). The Japanese commercial product of dried fruiting bodies from another strain of A. brasiliensis, cultivated indoors in Japan, was also purchased and tested as another indoor-cultivated sample. Outdoor- and indoor-cultivated A. brasiliensis KA21, and the indoor-cultivated Japanese sample were named KAOD, KAID, and JAID, respectively. The ITS-5.8S rDNA sequence of KAOD and JAID was analyzed using ITS4 and ITS5 primers at Techno-Suruga Laboratory Co., Ltd. (Shizuoka, Japan). KAOD and JAID showed 99.6% and 100% homology with A. subrubescens (GenBank accession number AY818654.1 and KJ541796.1, respectively).

Measurement of ingredients

Japan Food Research Laboratories (Shibuya, Tokyo) measured the ingredients of A. brasiliensis fruiting bodies using the standard protocols recommended by the Resources Council, the Science and Technology Agency of Japan.

Antioxidant assay

The ability of A. brasiliensis to scavenge DPPH free radicals was assessed using the previously described decolorization method, with minor modifications [16,17]. Briefly, powdered mushrooms were extracted with 50% methanol (50 mg/mL) at 60°C for 60 min. Trolox dissolved in 50% methanol was used as a standard antioxidant. Diluted extracts (20 μL) were combined with 200 μL of 150 μmol/mL...
samples was controlled for by using a blank, and the data was expressed as equivalents of Trolox.

### Feed preparation

Various doses of powdered fruiting bodies from *A. brasiliensis* were mixed into the basic synthetic diet AIN-93G (Oriental Yeast Co., Ltd, Tokyo, Japan), and cornstarch was substituted for *A. brasiliensis*. The doses of KAOD tested were 1, 3, and 10%, whereas KAID and JAID were tested at 3 and 10%, respectively. AIN-93G supplemented with cornstarch was used as the control diet. All feeds were admixed by Oriental Yeast Co., Ltd, and the molding and drying processes were carried out at 60°C or lower. Each test diet was irradiated with 30 kGy γ-rays for sterilization. The ingredients in the solid feed are shown in Table 1. To evaluate the heat stability of KAOD, AIN-93G and KAOD-10% were autoclaved and air-dried at 50°C, and named heat-treated AIN-93G and heat-treated KAOD, respectively.

### Experimental protocol

For the chemical hepatic injury experiments, mice were divided randomly into 13 groups. All groups of mice were fed an experimental diet for 11 days. On the 10th day, hepatotoxicity was induced by intraperitoneal (i.p.) injection of CCl₄ (4 ml/kg body weight of 1% CCl₄ solution in olive oil). In the control group, mice received an i.p. injection of olive oil alone. The feeding design is

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**Table 1 Ingredients in solid feed**

| Ingredient                  | Content (g/kg diet) |
|-----------------------------|---------------------|
| Corn starch                 | 397.486 R           |
| Casein                      | 200.000             |
| α-Corn starch               | 132.000             |
| Sucrose                     | 100.000             |
| Soybean oil                 | 70.000              |
| Cellulose powder            | 50.000              |
| AIN-93G Mineral Mix         | 35.000              |
| AIN-93G Vitamin Mix         | 10.000              |
| L-Cystine                   | 3.000               |
| Choline bitartrate          | 2.500               |
| tert-butylhydroquinone       | 0.014               |
| KAOD, KAID, or JAID         | R                   |

Control diet (AIN-93G): R =0.
KAOD-1%: R =10.
KAOD-3%: R =30.
KAOD-10%: R =100.
KAID-3%: R =30.
JAID-10%: R =100.

DPPH in 50% methanol. All samples were prepared in triplicate. Following incubation in the dark at room temperature for 30 min, the absorbance at 517 nm was read using a Safire microplate reader (Tecan, Salzburg, Austria). The effect of natural color changes of the

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![Figure 1 Experimental protocol.](http://www.biomedcentral.com/1472-6882/14/454)
shown in Figure 1. Mice in groups I and II received AIN-93G and KAOD-10%, respectively, during the experiment, and were treated with olive oil. Mice in groups III–VI received AIN-93G, KAOD-10%, KAOD-3%, and KAOD-1%, respectively. Mice in Groups VII and VIII received AIN-93G, and then received KAOD-10% on day 9 until the end of the experiment. Mice in Groups IX or XI were fed AIN-93G, and then received KAOD-10% for 24 h before or after the injection of CCl₄, respectively. Mice in Groups XII or XIII received AIN-93G, followed by heat-treated AIN-93G or heat-treated KAOD, respectively, from day 7 until the end of the experiment. All mice in Groups III–XIII were treated with CCl₄ on day 10. Twenty-four hours after the i.p. administration of CCl₄ or olive oil, mice were sacrificed by CO₂ inhalation. Blood samples were collected by heart puncture, and the liver was removed and used to analyze biochemical markers of early acute hepatic damage and for histopathological evaluation.

Measurement of serum ALT, AST, and LDH activities

Collected whole blood was allowed to stand at room temperature for 90 min, and then centrifuged (12,000 rpm for 10 min at 4°C) to obtain serum. The enzyme activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum were then measured using a Wako Transaminase CII-Test kit (Wako Pure Chemical Co.) following the manufacturer’s instructions. The color development was stopped using citric acid solution, and the optical density at 550 nm was measured using a microplate reader (MTP450; Corona Electric, Ibaraki, Japan).

Lactate dehydrogenase (LDH) activity was assayed according to the instructions supplied with the commercial assay kit (NescoVL LD, Alfresa Pharma Co., Osaka, Japan). The absorbance at 340 nm was read using a Safire microplate reader (Tecan).

Histopathological analysis

Livers were fixed in 10% formalin neutral buffered solution (pH 7.4) (Wako Pure Chemical Co.), embedded in paraffin blocks, and thinly sectioned. Tissue sections were stained

Table 2 Basic nutrient composition comparison of cultivation methods

|          | KAOD  | KAID  | JAID  | Unit |
|----------|-------|-------|-------|------|
| Moisture | 7.5 g | 5.5 g | 5.9 g | g    |
| Energy   | 179 kcal | 184 kcal | 183 kcal | kcal |
| Protein  | 39.8 g | 43.3 g | 41.6 g | g    |
| Fat      | 2.9 g | 3.7 g | 3.1 g | g    |
| Carbohydrate | 24.2 g | 21.5 g | 20.2 g | g    |
| Fiber    | 18.9 g | 19 g | 22.7 g | g    |
| Sodium   | 3.4 g | 7.5 g | 9.7 g | mg   |
| Vitamin A (total caronene) | - | - | - | - |
| Vitamin B₁ (Thiamin) | 0.79 mg | 1.41 mg | 0.52 mg | mg |
| Vitamin B₂ (Riboflavin) | 3.5 mg | 4.01 mg | 5.49 mg | mg |
| Vitamin B₆ | 0.49 mg | 0.87 mg | 0.76 mg | mg |
| Vitamin B₁₂ | 0.27 μg | - | - | μg |
| Niacin   | 48.8 mg | 47.7 mg | 53.4 mg | mg |
| Pantothenic acid | 20.2 mg | 23.9 mg | 15.9 mg | mg |
| Folic acid | 0.18 mg | 0.36 mg | 0.21 mg | mg |
| Biotin   | 150 μg | 174 μg | 128 μg | μg |
| Total vitamin C (Total c acid) | - | - | - | - |
| Vitamin D | 30.7 μg | - | 0.9 μg | μg |
| Vitamin E (Total tocopherol) | - | - | - | μg |
| Vitamin K₁ (Phylloquinone) | - | - | - | μg |
| Calcium  | 42.5 mg | 36.6 mg | 1.6 mg | mg |
| Iron     | 11 mg | 11.8 mg | 7.76 mg | mg |
| Phosphorus | 994 mg | 987 mg | 1110 mg | mg |
| Magnesium | 98.9 mg | 105 mg | 114 mg | mg |
| Potassium | 2.84 g | 2.96 g | 2.67 g | g |
| Copper   | 16.5 mg | 12.3 mg | 1.65 mg | mg |
| Iodine   | - | - | - | - |
| Manganese | 0.67 mg | 0.7 mg | 0.82 mg | mg |
| Selenium | 51 μg | 120 μg | 24 μg | μg |
| Zinc     | 10.9 mg | 19.7 mg | 16.4 mg | μg |
| Total chromium | 0.05 mg | - | - | mg |
| Molybdenum | - | - | - | mg |

*; not detected.

All data are shown for 100 g dry weight, and were measured by Japan Food Research laboratories.

Figure 2 Free radical scavenging activities of various A. brasiliensis. The antioxidant capacities of KAOD, KAID, and JAID extracts (powder 50 mg/mL, supernatant) were measured using the DPPH radical-decolorization assay. Each sample was diluted and reacted with the DPPH reagent. Results are presented as mean (n=3) ± SD. Significant difference from KAOD extract: ***p <0.001.
with hematoxylin and eosin (HE). The Biopathology Institute Co., Ltd. (Oita, Japan) prepared paraffin blocks and carried out HE staining. The sections were then observed under a normal light microscope (Biozero BZ-8100 microscope, Keyence, Osaka, Japan).

Statistical analysis
The significance of the differences between the means was assessed by Student’s t-test.

Results
Influence of cultivation conditions of the antioxidative activities of A. brasiliensis
We demonstrated previously that the enzymatic activities of A. brasiliensis fruiting bodies can be modulated by cultivation conditions, such as open-air or indoor growth [15]. Therefore, we first compared the ingredients in the fruiting bodies of A. brasiliensis and studied the antioxidant activities of KAOD, KAID, and JAID. As shown in Table 2, the basic composition of KAOD and JAID was similar (except for vitamin D and calcium), and there were no major differences between KAOD and KAID. Nevertheless, in the DPPH radical-scavenging assay, the antioxidant activity of KAOD extract was higher than that of KAID or JAID (Figure 2). These data suggest that outdoor cultivation augments the antioxidant capacity of A. brasiliensis fruiting bodies.

Effect of outdoor cultivated A. brasiliensis on CCl4-induced hepatic injury
Because KAOD yielded increased antioxidative activity, we next focused on its pharmacological activity. To evaluate whether the oral administration of A. brasiliensis whole fruiting bodies exerted hepatoprotective properties, we carried out an in vivo experiment using a mouse model of CCl4-induced hepatitis. As shown in Figure 3A, although KAOD-1% did not suppress the blood release of liver enzymes, 10 days of administration of KAOD-10%

Figure 3 Hepatoprotective effects of KAOD. Serum and livers were isolated from ICR mice fed KAOD-1%, KAOD-3%, or KAOD-10% ad libitum for 11 days. (A) ALT, AST, and LDH activities in the serum from mice fed (a) KAOD-1% and injected with CCl4, (b) KAOD-3% and injected with CCl4 or (c) KAOD-10% and injected with CCl4 or olive oil were measured using commercially available kits. Data are presented as mean ± standard deviation, (a) n = 6, (b) n = 12, (c) n = 7 (CCl4), n = 3 (olive oil). Significant difference from AIN-93G: **p < 0.01; ***p < 0.001; n.s.: not significant. (B) HE-stained liver sections from mice administered with (a-1) AIN-93G with CCl4 (control for KAOD-1%), (a-2) KAOD-1% with CCl4, (b-1) AIN-93G with CCl4 (control for KAOD-3%), (b-2) KAOD-3% with CCl4, (c-1) AIN-93G with CCl4 (control for KAOD-10%), (c-2) KAOD-10% with CCl4, (c-3) AIN-93G with olive oil, or (c-4) KAOD-10% with olive oil were observed under a normal light microscope. Scale bar =100 μm.
or KAOD-3% significantly inhibited CCl₄-induced changes in the circulating levels of enzymes including ALT, AST, and LDH. In particular, mice fed KAOD-10% showed comparable levels of biochemical markers as olive oil-injected mice. Data obtained from histopathological studies supported the results of the biochemical analyses. (Figure 3B). Liver sections from control and KAOD-alone treated mice showed normal liver architecture. Injection with CCl₄-induced obvious liver injury, which presented as large areas of extensive necrosis and a loss of hepatic architecture. KAOD prevented the development of CCl₄-induced histological changes in a dose-dependent manner.

The effect of cultivation conditions of the hepatoprotective activities of A. brasiliensis

The above experiments defined the optimal dose of KAOD for hepatoprotection, therefore, we next prepared experimental diets containing KAID and compared its effects on outdoor- and indoor-cultivated A. brasiliensis KA21. Although KAOD-3% exerted hepatoprotective effects, KAID-3% did not inhibit the release of hepatic enzymes or prevent histopathological changes in the liver (Figure 4).

To confirm these effects, we prepared a commercially available product from another strain of A. brasiliensis cultivated indoors in Japan, and mixed it into the experimental diet as JAID-10%. Oral administration of JAID-10% did not suppress the acute liver injury induced by injection of CCl₄ (Figure 4). These results strongly suggest that indoor cultivation significantly reduces the hepatoprotective activity of A. brasiliensis fruiting bodies.

Optimization of the hepatoprotective properties of A. brasiliensis

To determine the optimal use of A. brasiliensis, we assessed the length of time taken for orally administered KAOD-10% to protect against CCl₄-induced liver injury. Administration of KAOD-10% for 2 days was sufficient for hepatoprotective effects, assessed by the suppression of enzyme alterations (Figure 5A). Moreover, to assess whether the hepatoprotective effects of A. brasiliensis

![Figure 4 Effect of cultivation conditions of the hepatoprotective activities of A. brasiliensis.](image-url)
were preventive or curative, mice were treated with KAOD-10% for 24 h before or after injection with CCl₄. Hepatoprotection was observed only in the pre-ingestion group. In addition, histopathological analyses strongly supported a hepatoprotective effect of KAOD-10% after 24 h of administration in the pre-ingestion group (Figure 5). These data suggest that the oral administration of KAOD exerts preventative effects against acute hepatic injury, and applies rapid effects.

During the preparation of experimental diets containing KAOD, we avoided high temperatures to preserve a variety of heat-sensitive properties. Therefore, we next assessed the effects of heat processing on the hepatoprotective properties of A. brasiliensis. As shown in Figure 6, the hepatoprotective effects of orally administered KAOD-10% on the release of hepatic enzymes and the histopathological changes in the liver were not affected by autoclaving, suggesting that the hepatoprotective properties of A. brasiliensis are heat stable.

**Discussion**

In 2008, a clinical study reported that A. brasiliensis extracts normalized liver function in hepatitis B patients [18]. In addition, several animal experiments have investigated the hepatoprotective effects of these mushroom extracts [19,20]. However, the process of extraction not only concentrates the active ingredients, but also increases the potential for side effects. In 2006, possible adverse effects of A. brasiliensis were reported, where it was suggested that intake of these mushroom extracts may induce hepatic dysfunction [21]. Therefore, in this study we did not make extracts, and demonstrated the hepatoprotective activities of non-extracted fruiting bodies of A. brasiliensis using outdoor cultivation (Figures 3). In addition, because indoor-cultivated mushrooms failed to protect against liver damage (Figure 4), these mushrooms may require extraction or higher doses to exert hepatoprotective activities, which may increase the possibility of adverse effects. The safety of the normal use of
non-extracted KAOD was demonstrated in our previous clinical study [6]. Therefore, outdoor cultivation could be a better method for reducing the risk of side effects. In addition, because hepatoprotective activities of KAOD were observed even when it was administered 24 h before CCl\textsubscript{4} injection (Figure 5) and was not affected by autoclaving (Figure 6), the key molecules for hepatoprotection could be rapid-acting and heat-tolerant. Thus, these characteristics could be exploited to help use this mushroom for inhibiting hepatic damage.

Although indoor systematic cultivation has the advantage of being able to provide a stable supply, we believe that outdoor growth, and particularly sunshine, is one of the most important factors for the biological activity of this mushroom. Sunlight increased the levels of ingredients such as vitamin D and β-glucan, and enhanced the activity of several enzymes [15]. This study showed that although outdoor-cultivated \textit{A. brasiliensis} contained more abundant β-glucan than that grown in the shade, the immunomodulatory effects of these mushrooms may be similar because the primary structure of β-glucan was unchanged. Nevertheless, many reports have suggested beneficial effects of vitamin D [22,23]. In addition, other biological properties including enzymes and molecules produced by the enzymes could be affected by the cultivation conditions. Consistent with this, our study demonstrated that KAOD yields increased antioxidative activity compared with other mushrooms (Figure 2). Therefore, it is possible that outdoor cultivation could augment the antioxidative properties of \textit{A. brasiliensis}, because the mushrooms protect themselves from sunlight-induced oxidative damage.

Many reports have described hepatoprotective properties of \textit{A. brasiliensis} [24,25], but the underlying mechanism(s) of these effects are yet to be elucidated. Because both JAID-10% and KAOD-1% failed to exert hepatoprotection, we considered if KAOD contained factors that would explain the 10-fold greater hepatoprotective properties than JAID. However, the basic ingredients of KAOD, KAID, and JAID were similar, except for vitamin D (Table 2), and we failed to demonstrate any contribution of vitamin D2 to hepatoprotection (data not shown). To clearly define the pharmacologic actions of \textit{A. brasiliensis}, the molecular mechanism(s) behind the hepatoprotective effects of this mushroom must also be investigated.

Our current research focuses on the antioxidative components of \textit{A. brasiliensis}, because the oral administration...
of several antioxidants protects against liver injury in animal models [26,27]. Therefore, we will attempt to identify the key molecules that exert these hepatoprotective activities using both indoor- and outdoor-cultivated Agaricus brasiliensis in future studies. The antioxidant activity of this mushroom is modulated not only by cultivation method, but also by different maturation phases [17]. Therefore, we propose that the pharmaceutical activity of Agaricus brasiliensis could be optimized by the development of a basic manufacturing method.

Conclusions

In the present study, we demonstrated that open-air cultivation strongly upregulated the total antioxidant activity of Agaricus brasiliensis. In addition, outdoor-cultivated Agaricus brasiliensis was more effective than mushrooms grown in the shade at protecting against CCl4-induced acute hepatic injury in mice. Therefore, we concluded that open-air cultivation could augment the antioxidative and hepatoprotective properties of the fruiting bodies of Agaricus brasiliensis.

Abbreviations

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; DPPH: 1,1-diphenyl-2-picrylhydrazyl; HE: Hematoxylin and eosin; LDH: Lactate dehydrogenase; TLR: Toll-like receptor.

Competing interests

The authors declare that they have no competing interests.

Authors’ contributions

All authors conceived of the study, and supervised the data collection. DY led the statistical analysis. All authors participated in drafting and revising the manuscript, and all authors approved the final manuscript.

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References

1. Wasser SP, Didukh MY, de A Amazonas MLA, Nevo E, Stamets P, da Eira AF: Is a widely cultivated culinary-medicinal royal Sun Agaricus (the hemmatsukatemushroom) indeed Agaricus blazei murrill? Int J Med Mushrooms 2002, 4(4):267–290.
2. Kertjan RW: Agaricus subrufescens, a cultivated edible and medicinal mushroom, and its synonyms. Mycologia 2005, 97(1):12–24.
3. Wistrassamekong K, Kunarathana SC, Thongklang N, Zhao R, Callac P, Moukha S, Ferandon C, Chuleitine C, Hyde KD: Agaricus subrufescens: a review. Saudi J Biol Sci 2012, 19(2):131–146.
4. Boulge G, Nishitani Y, Shiohmi H, Yoshida M, Azuma T, Hashimoto T, Kanazawa K, Mizuno M: Oral treatment with extract of Agaricus blazei murrill enhanced Th1 response through intestinal epithelial cells and suppressed OVA-sensitized allergy in mice. Evid Based Complement Alternat Med 2011, 2011:332180.
5. Ellertsen LK, Hetland G: An extract of the medicinal mushroom Agaricus blazei Murill can protect against allergy. Clin Mol Allergy 2009, 7:6.
6. Liu Y, Fukuswtani Y, Okumura K, Takeda K, Ishibashi KI, Furukawa M, Ohno N, Mori K, Gao M, Motoi M: Immunomodulating activity of Agaricus brasiliensis KA21 in mice and in human volunteers. Evid Based Complement Alternat Med 2008, 5(2):205–219.
7. Ohno N, Akanuma AM, Miura NN, Adachi Y, Motoi M: 1-(3–3)-beta-D-glucan in the fruit bodies of Agaricus blazei. Pharm Pharmacol Lett 2001, 11(2):87–90.
8. Ohno N, Furukawa M, Miura NN, Adachi Y, Motoi M, Yadoœae T: Antitumor beta-glucan from the cultured fruit body of Agaricus blazei. Bioll Pharm Bull 2001, 24(7):820–828.
9. Yamanaka D, Motoi M, Ishibashi K, Miura NN, Adachi Y, Ohno N: Effect of Agaricus brasiliensis-derived cold water extract on Toll-like receptor 2-dependent cytokine production in vitro. Immunopharmacol Immunotoxicol 2012, 34(4):561–570.
10. Kasi H, He LM, Kawamura M, Yang PT, Deng WX, Munkanta M, Yamashita A, Terunuma H, Hirama M, Hotuchi I, Natori T, Koga T, Amano Y, Yamaguchi N, Ito M: IL-12 Production induced by Agaricus blazei fraction H (ABH) involves toll-like receptor (TLR). Evid Based Complement Alternat Med 2004, 1(3):259–267.
11. Yamanaka D, Tada R, Adachi Y, Ishibashi K, Motoi M, Iwakura Y, Ohno N: Agaricus brasiliensis-derived beta-glucans exert immunoenhancing effects via a T-cell-dependent patheway. Int Immunopharmacol 2012, 14(3):311–319.
12. Chang JB, Wu MF, Yang YY, Leu SJ, Chen YL, Yu CS, Yu CC, Chang SJ, Lu HF, Chung JG: Carbon tetrachloride-induced hepatotoxicity and its amelioration by Agaricus blazei Murrill extract in a mouse model. In Vivo 2011, 25(6):971–976.
13. AI-Dibas AM, AI-Daihan SK, Bhat RS: Agaricus blazei Murrill as an efficient hepatoprotective and antioxidant agent against CCl4-induced liver injury in rats. Saudi J Biol Sci 2012, 19(3):303–309.
14. Yuan LP, Chen FH, Ling L, Dou PF, Bo H, Zhong MM, Xia LJ: Protective effects of total flavonoids of Bidens pilosa L. (TFB) on animal liver injury and liver fibrosis. J Ethnopharmacol 2008, 116(3):539–546.
15. Hashimoto S, Akanuma AM, Motoi M, Imai N, Rodrigues CA, Namera S, Miura NN, Adachi Y, Ohno N: Effect of culture conditions on the chemical composition and biological activities of Agaricus brasiliensis S. Wasser et al. (Agaricomycetidae). Int J Med Mushrooms 2006, 8(4):329–341.
16. Böös MS: Antioxidant determinations by the use of a stable free radical. Nature 1958, 181(4671):1199–1200.
17. Moutas F, Harase Umezoe S, Seko Takemura O, Andrea Linde G, Barros Calato N: Antioxidant Activity of Agaricus brasiliensis Basidiocarps on Different Maturation Phases. Braz J Microbiol 2011, 42(1):197–202.
18. Hsu CH, Hwang KC, Chiang YH, Chou P: The mushroom Agaricus blazei Murill extract normalizes liver function in patients with chronic hepatitis B. J Altern Compliment Med 2008, 14(4):299–301.
19. Wu MF, Hsu YM, Tang MC, Chen HC, Chung JS, Lu HF, Lin JP, Tang NY, Yeh C, Yeh MY: Agaricus blazei Murrill extract abrogates CCl4-induced liver injury in rats. In Vivo 2011, 25(5):135–40.
20. Zhang C, Han C, Zhao B, Yu H: The protective effects of aqueous extracts of wild-growing and fermented Royal Sun mushroom, Agaricus brasiliensis S. Wasser et al. (higher basidiomycetes), in CCl4-induced oxidative damage in rats. Int J Med Mushrooms 2012, 14(6):557–561.
21. Muki H, Watanabe T, Ando M, Katsunuma N: An alternative medicine, Agaricus blazei, may have induced severe hepatic dysfunction in cancer patients. Jpn J Clin Oncol 2006, 36(12):808–810.
22. Holick MF: Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. Am J Clin Nutr 2004, 80(6 Suppl):1678S–1688S.
23. Martineau AR, Wilkinson RJ, Wilkinson KA, Newton SM, Kampmann B, Hall RM, Packe GE, Davidson RN, Eldridge SM, Maunsell ZJ, Rainbill SJ, Berry JL, Griffiths CJ: A single dose of vitamin D enhances immunity to mycobacteria. Am J Respir Crit Care Med 2007, 176(2):208–213.
24. Saad EA: Curative and protective effects of L-arginine on carbon tetrachloride-induced hepatotoxicity in mice. Biochem Biophys Res Commun 2012, 423(1):147–151.
25. Jiang W, Gao M, Sun S, Bi A, Xin Y, Han X, Wang L, Yin Z, Luo L: Protective effect of L-theanine on carbon tetrachloride-induced acute liver injury in mice. Biochem Biophys Res Commun 2012, 422(2):344–350.

26. Bai X, Qu A, Guan J, Shi Z: Antioxidant and protective effect of an oleanolic acid-enriched extract of A. deliciosa root on carbon tetrachloride induced rat liver injury. Asia Pac J Clin Nutr 2007, 16(Suppl 1):169–173.

27. Xia D, Fan Y, Zhang P, Fu Y, Ju M, Zhang X: Protective effects of the flavonoid-rich fraction from rhizomes of Smilax glabra Roxb. on carbon tetrachloride-induced hepatotoxicity in rats. J Membr Biol 2013, 246(6):479–485.

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