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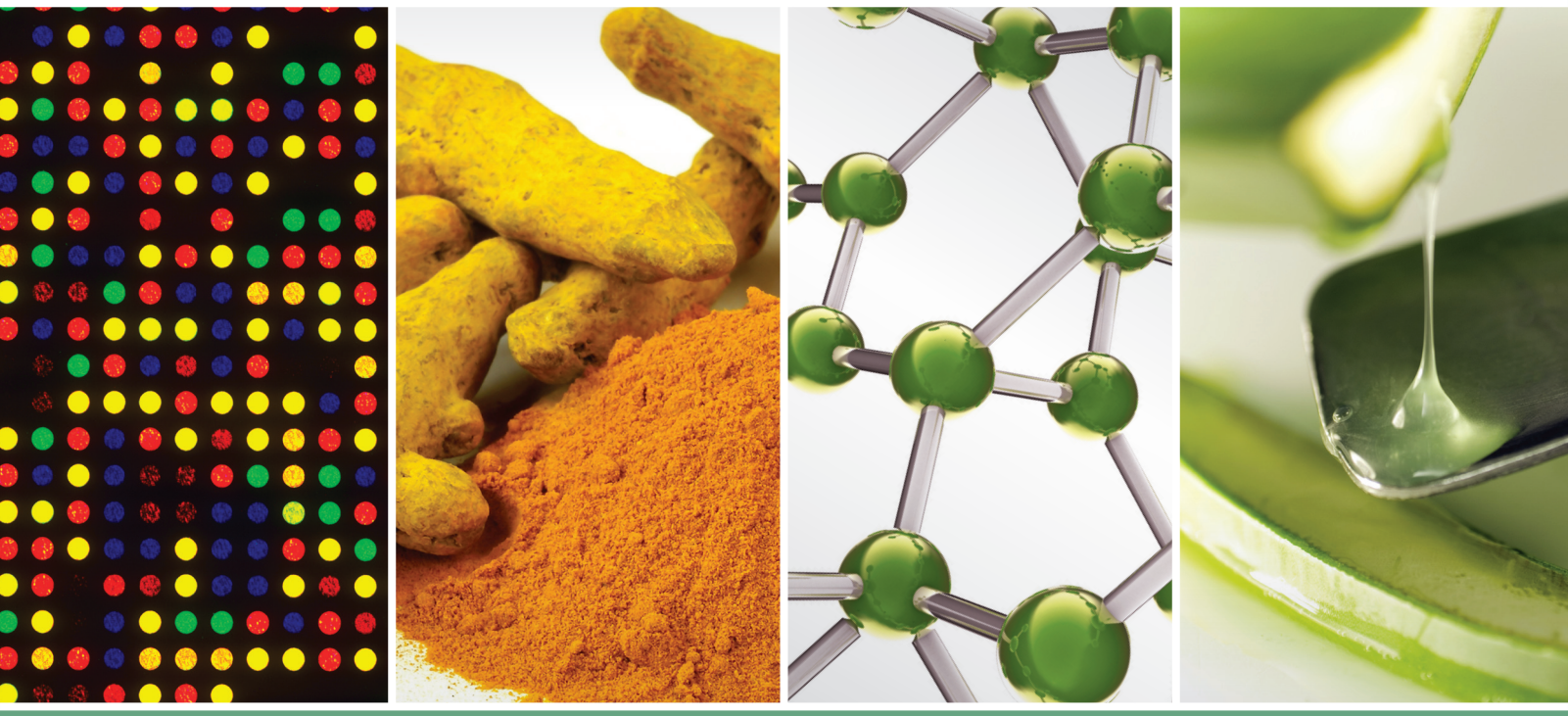
Guest Editors: Chunchao Han, John E. Smith, Jianyou Guo, Marie Foulongne-Oriol, and Bo Cui



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# Editorial Board

# Contents

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2014, Article ID 638561, 2 pages
http://dx.doi.org/10.1155/2014/638561

*Editorial*

# Medicinal and Edible Fungi as an Alternative Medicine for Treating Age-Related Disease

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Received 25 March 2014; Accepted 25 March 2014; Published 29 April 2014



Age-related diseases such as type 2 diabetes, cardiovascular disease, and cancer involve epigenetic modifications, where accumulation of minute changes in the epigenome over time leads to disease manifestation. Epigenetic changes are influenced by life style and diets. This represents an avenue whereby dietary components could accelerate or prevent age-related diseases through their effects on epigenetic modifications. Therefore, new therapeutic approaches are needed to treat them more efficiently.

The lack of effective and widely applicable pharmacological treatments for ischemic stroke patients may explain a growing interest in traditional medicines. Some of basidiomycetous fungi produce substances with potential medical effects and are called medicinal mushrooms. Fungi (yeasts, molds, and mushrooms) have diverse morphological, physiological, and ecological characteristics that support their diverse lifestyles. These specific interspecies interactions depend on the production of a wide range of bioactive substances. Higher fungi are well recognized for their medicinal properties and have been used in traditional medicine for millennia. The medicinal effects attributed to fungi, based mainly on uncharacterized substances or extracts, include antiviral, immunomodulatory, antitumor, antioxidant, radical scavenging, anti-inflammatory, antihyperlipidemic or antihypercholesterolemic, hepatoprotective, and antidiabetic effects.

*Cordyceps sinensis* (CS) has been used as a tonic for longevity, endurance, and vitality for thousands of years by the Chinese. Many studies have shown that CS regulates insulin sensitivity and decreases plasma cholesterol levels. W. Qi et al. investigated the protective effect of cordymin on diabetic osteopenia in alloxan-induced diabetic rats and the possible mechanisms involved. The diabetic rats received daily intraperitoneal injection with cordymin (20, 50, and 100 mg/kg/day) for 5 weeks. Cordymin could restore the circulating blood glucose, glycosylated hemoglobin (HbA1c), serum alkaline phosphatase (ALP), tartrate resistant acid phosphatase (TRAP), and insulin levels in a dose-dependent manner. Also, the treatment of diabetic rats with cordymin could partially reverse the $\beta$ cells death and decrease the total antioxidant status (TAOS) in the diabetic rats. The results may directly and indirectly account for the possible mechanism of the beneficial effect of cordymin on diabetic osteopenia.

The basidiomycete *Agaricus blazei* Murill (AbM), popularly known as "sun mushroom," is native to Brazil and widely grown in Japan and China because of its medicinal properties. It is widely used for nonprescript, medicinal purposes, both as an edible mushroom and in the form of extracts. AbM has traditionally been used for the prevention of a range of diseases, including cancer, hepatitis, atherosclerosis, hypercholesterolemia, diabetes, and dermatitis. P. Wang et al. investigated the anti-inflammatory activity of water-soluble polysaccharide of *Agaricus blazei* Murill (WSP-AbM) on ovariectomized osteopenic rats. The rats were administered orally WSP-AbM (200 mg/kg BW) for 8 weeks. Subsequent serum maleic dialdehyde (MDA) level, total antioxidant status (TAOS), nuclear factor kappa B (NF-$\kappa$B) level, polymorphonuclear (PMN) cells level, interleukin-1$\beta$ (IL-1$\beta$) level, inducible nitric oxide synthase (iNOS) level, tumor necrosis factor-$\alpha$ (TNF-$\alpha$) level, adhesion molecule (ICAM-1),

and cyclooxygenase-2 (COX-2) were determined by enzyme linked immunosorbent assay (ELISA) and immunohistochemistry, respectively. WSP-AbM administration markedly decreased serum IL-1$\beta$ and TNF-$\alpha$ levels and the expressions of ICAM-1, COX-2, and iNOS NF-$\kappa$B compared with OVX rats. WSP-AbM administration also markedly decreased PMN infiltration. In conclusion, we observed that WSP-AbM supplementation had anti-inflammatory effects in a model of osteoporosis disease. H. Wang et al. also review *Agaricus blazei* Murill. They demonstrated ABM is useful against a variety of diseases like cancer, tumor, chronic hepatitis, diabetes, atherosclerosis, hypercholesterolemia, and so on. W. Ji et al. examined the neuroprotective effect of *Agaricus brasiliensis* (AbS) against STZ-induced diabetic neuropathic pain in laboratory rats. After 6 weeks of treatments, all animals were sacrificed to study various biochemical parameters. Treatment with AbS 80 mg/kg in diabetic animals showed significant increase in body weight, pain threshold, and paw withdrawal threshold and significant decrease in serum glucose, LPO and NO level, Na-K-ATPase level, and TNF-$\alpha$ and IL-1$\beta$ level as compared to vehicle treated diabetic animals in dose and time-dependent manner. AbS can offer pain relief in PDN.

*Ganoderma lucidum* is a popular medicinal mushroom that has been used as a home remedy in traditional Chinese medicine for the prevention or treatment of a variety of diseases including cancer. Today, *G. lucidum* is recognized as a dietary supplement recommended in many countries as a cancer therapeutic. Another important property of this edible mushroom is the ability to take up and accumulate trace metals such as cadmium, lead, arsenic, copper, nickel, silver, chromium, and mercury in the body or mycelium of the mushroom. G. Min-chang et al. compared the effect and toxicity of organic selenium (selenium-enriched protein from *Ganoderma lucidum*) with inorganic selenium (IOSe) in preventing asthma in ovalbumin-induced asthmatic mice. The results showed that the serum Se levels in the mice fed the Pro-Se were significant elevations. It results in restoration of the level of endogenous antioxidant enzyme, lower levels of TNF-$\alpha$ and IL-1$\beta$, and activated NF-$\kappa$B in the asthmatic mice. Our experiments have demonstrated profound differences between the activities of organic selenium and inorganic selenium in experimental conditions. These data provide an important proof of the concept that organic selenium might be a new potential therapy for the management of childhood asthma in humans.

By compiling these papers, we hope to enrich our readers and researchers with respect to medicinal and edible fungi, yet usually highly treatable fungi for age-related disease.

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Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2014, Article ID 182817, 6 pages
http://dx.doi.org/10.1155/2014/182817

*Research Article*

# Effects of Selenium-Enriched Protein from *Ganoderma lucidum* on the Levels of IL-1$\beta$ and TNF-$\alpha$, Oxidative Stress, and NF-$\kappa$B Activation in Ovalbumin-Induced Asthmatic Mice

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Received 31 October 2013; Accepted 23 December 2013; Published 10 February 2014

Academic Editor: Chunchao Han



The purpose of this study was to compare the effect and toxicity of organic selenium (Pro-Se) with inorganic selenium (IOSe) in preventing asthma in ovalbumin-induced asthmatic mice. After the mice were treated orally with Pro-Se and IOSe, respectively, the plasma Se levels, Se accumulation in liver and kidney, tumor necrosis factor alpha (TNF-$\alpha$), interleukin 1 beta (IL-1$\beta$), oxidative stress, and NF-$\kappa$B activation in lung were examined. The results showed that the serumal Se levels in the mice fed the Pro-Se were significant ($P < 0.01$) elevations. It results in restoration of the level of endogenous antioxidant enzyme, lower levels of TNF-$\alpha$ and IL-1$\beta$, and activated NF-$\kappa$B in the asthmatic mice. Our experiments have demonstrated profound differences between the activities of organic selenium and inorganic selenium in experimental conditions. These data provide an important proof of the concept that organic selenium might be a new potential therapy for the management of childhood asthma in humans.

## 1. Introduction

Asthma is a chronic disease associated with airway hyperresponsiveness, airway obstruction, and airway remodelling [1, 2]. The principal pathophysiology of asthma is chronic inflammation of the lower respiratory tract [3]. Anti-inflammatory agents such as inhaled steroids and leukotriene receptor antagonists along with long acting bronchodilators are the mainstay of asthma pharmacotherapy. However, potential long term side effects, prohibitive costs, and suboptimal adherence to asthma medications are ongoing challenges to optimal asthma control. Treatment options are, therefore, quite limited for asthma and the need to search for other therapies has been recognized by many experts in the field [4, 5].

The trace element selenium (Se) is an essential nutrient for all mammalian species and is of fundamental importance for human biology. Deficient Se intake may dramatically affect inflammation and immune responses. Also, the use of Se supplementation to increase Se status to supraphysiological levels may be exploited to modulate immune processes that drive certain health disorders, such as the T helper 2 (Th2) responses that drive allergic asthma [6, 7]. Therefore, Se intake has been hypothesized to play a role in the development and/or severity of this complex disease, asthma.

However, Mahan and Kim [8] suggested that Se may not be as biologically effective as the Se indigenous in grains, which is primarily in the organic form of selenomethionine. Mahan and Parrett [9] found that total Se excretion decreased and tissue retention increased when an organic, rather than an inorganic, source of Se was fed. Further, organic selenium is reported to be better absorbed, has higher bioavailability, and is less toxic than inorganic selenium [10–12]. In the present study, we isolated selenium-contained protein (Pro-Se) with different selenium content, indicated the organic selenium, from the *Ganoderma lucidum*, and compared the effect and toxicity of organic selenium in preventing asthma with those of inorganic selenium (IOSe).

## 2. Materials and Methods

*2.1. Chemicals.* Sodium selenite was purchased from Anhui Star New Material Technology Co., Ltd., China.

*2.2. Sodium Selenite Solution (IOSe).* Sodium selenite was dissolved in normal saline. An ampule was filled with 0.4 mL of IOSe with 0.104 $\mu$g sodium selenite.

*2.3. Selenium-Contained Protein (Pro-Se).* *Ganoderma lucidum* was cultured on the medium which was composed of peptone (10.0 g/L), beef extract (10.0 g/L), yeast extract (5.0 g/L), $K_2HPO_4$ (2.0 g/L), triammonium citrate (2.0 g/L), sodium acetate (5.0 g/L), glucose, tween 80 (1.0 mL/L), $MgSO_47H_2O$ (0.58 g/L), $MnSO_4H_2O$ (0.25 g/L), corn steep liquor (3.0 g/L), and cysteine hydrochloride (0.3 g/L). After 6 h, sodium selenite of different concentrations (0, 2.5, 5.0 mg/mL) was added to the medium. The Se-enriched *Ganoderma lucidum* were marked with Se0, Se2.5, and Se5.0, respectively, according to the sodium selenite concentration. The Se-enriched *Ganoderma lucidum* was removed by centrifugation after cultured with sodium selenite for another 12 h, and then it was preserved in the dark at room temperature after freeze drying (−50°C, under vacuum).

The protein was isolated according to the method of Kadrabova et al. [13]. 3.0 g of each kind of dry bacteria samples (Se0, Se2.5, and Se5.0) was dissolved in cold sodium hydroxide solution (0.25 M); the bacteria samples were broken by ultrasonication and incubated at 50°C in water bath for 2 h. The supernatant was obtained by filtration and the residue was repeated twice with 50 mL of NaOH (0.25 M). Then, ammonium sulfate was added to the supernatant to make 95% saturated solution which was followed by keeping it overnight at 4°C. Protein was precipitated using centrifuge at 6000 rpm for 30 min at 4°C. The resulting precipitate was then dissolved in 10.0 mL of Tris-HCl (pH 8.0, 50 mM). This solution was passed through 0.22 mm syringe filter and dialysed against 1.0 L of Tris-HCl (pH 8.0, 50 mM) using a membrane with 3500 molecular weight cutoff at 4°C three times to remove ammonium sulfate. Each kind of protein was marked with Pro-Se0, Pro-Se2.5, and Pro-Se5.0. The selenium content in each protein sample has been determined to be 0, 0.260, and 0.333 mg/L, respectively, by hydride generation-atomic absorption spectrometry.

*2.4. Animals.* Twenty-to-22-day-old female BALB/c mice, weighing from 12 g to 15 g, were obtained from the Experimental Animal Center of Zhejiang. Mice were housed with free access to food and water in a room with an ambient temperature of 22 ± 2°C and a 12 : 12 h light/dark cycle. All experiments were carried out in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The normal basal diet (NBD) for the mice was also purchased from the Center of Laboratory Animals, Zhejiang University. The NBD containing 0.05 $\mu$g/g of Se in a pellet form was formulated to meet the nutrient requirements for normal laboratory mice.

*2.5. Induction of Model of Asthma.* Mice were sensitized via 2 intraperitoneal injections of 10 $\mu$g of ovalbumin (grade V, ≥98% pure, Sigma, St. Louis, MO, USA) with alum adjuvant on days 0 and 14 of the experiment. Starting on day 21, the mice, housed in whole-body exposure chambers, were exposed to 1% aerosolized ovalbumin for 30 min a day, 3 days a week, for 9 weeks. The temperature was kept at 20°C to 25°C and the relative humidity at 40% to 60%.

*2.6. Experimental Design.* Mice were randomly assigned to six groups: control group with saline treatment, asthmatic group with saline treatment, and asthmatic group treated with 0.4 mL Pro-Se0 (0 $\mu$g selenium), 0.4 mL Pro-Se2.5 (0.104 $\mu$g selenium), 0.4 mL Pro-Se5.0 (0.1332 $\mu$g selenium), and 0.4 mL of IOSe (0.104 $\mu$g selenium). All animals surviving after 30 days were killed. The blood samples were drawn from orbital vein from all the groups and serum was separated for biochemical estimations. Tissue samples of lung, liver, and kidney were dissected from the visceral tissues. After washing with saline, the tissue samples were blotted dry and weighed. All samples were stored at −80°C for future analysis.

*2.7. Serum Selenium Measurement.* Plasma Se levels were determined by means of an atomic absorption spectrometer (AAS-3200, Shanghai, China). Plasma was diluted (1 : 3) with 0.05% Triton-X 100 and 0.05% antifoam B solution. $NiNO_3$ (0.2% w/w) was used as a modifier for Se measurement. All determinations were run in duplicate, and individual values were averaged. The highest standard addition concentration was 100 $\mu$g/L for Se determination. The autosampler system was used in the automix mode and with sample intake of the graphite tube of 1 $\mu$L/s. The total volume inserted in the tube was 20 $\mu$L (5 $\mu$L of matrix modifier, 10 $\mu$L of sample, and 5 $\mu$L of standard solution). Absorption readings were measured as peak height. The variation coefficient for replicate measurement was <3%. The lowest threshold was 10 $\mu$g/L for Se detection.

*2.8. Determination of Se Accumulation in Liver and Kidney.* Selenium concentrations were determined using atomic absorption (AA). Weighed aliquots of frozen tissues were digested in 3 stages: the first using 5 mL mixed acids (4 : 1 of nitric acid : perchloric acid), the second using a combination of 2 mL $HNO_3$ and 30% $H_2O_2$, and finally using 2 mL $HNO_3$. All digestions were performed at 130°C until the acid was completely evaporated and the residue dried before the next acid stage was started. After the third acid treatment, 1% $HNO_3$ was added to the digests and heated at 80°C for 1 h. After cooling, the sample volume was measured and analyzed.

*2.9. Estimation of Pro-Se on Oxidative Stress.* Serum was used for the assay of glutathione (GSH) content, Lipid peroxidation (LPO), glutathione peroxidase (GPx) activity, glutathione reductase (GR) activity, catalase (CAT) activity, $Na^+K^+$ATPase activity, and glutathione S transferase (GST) activity.

Table 1: The effect of Pro-Se and IOSe on selenium levels in serum ($n = 10$).

| Different groups | Selenium level ($\mu$g/mL) |
|---|---|
| Asthmatic group with saline | 0.48 ± 0.03 |
| Asthmatic group with Pro-Se5.0 | 0.79 ± 0.09** |
| Asthmatic group with Pro-Se2.5 | 0.61 ± 0.01* |
| Asthmatic group with Pro-Se0 | 0.34 ± 0.06 |
| Asthmatic group with IOSe | 0.53 ± 0.06 |
| Control group | 0.72 ± 0.03 |

*$P < 0.05$, **$P < 0.01$ versus saline-treated.

*2.10. Measurement of Pro-Se on IL-1β and TNF-α.* The concentration of interleukin-1$\beta$ (IL-1$\beta$) and TNF-$\alpha$ in the plasma was determined using a commercial ELISA kit (Shanghai Jinma Biological Technology Inc., China) according to the manufacturer's instructions.

*2.11. Measurement of Pro-Se on NF-κB Activation in Lung.* For measurement of NF-$\kappa$B activation in the lung, nuclear protein from lung samples was extracted using a commercial kit (Shanghai Jinma Biological Technology, Inc., China). Activation of NF-$\kappa$B in the lung was determined using an ELISA kit (Shanghai Jinma Biological Technology, Inc., China), following the manufacture's instruction. This kit specifically detects the p50 member of NF-$\kappa$B.

*2.12. Data Analysis.* Group means were compared by ANOVA with GraphPad Prism (GraphPad Software, Inc.). Multiple comparison tests were performed with Tukey for significant differences at $P < 0.05$.

## 3. Results

*3.1. The Effect of Pro-Se on Selenium Levels in Serum.* As shown in Table 1, Pro-Se significantly increased Se content in mice serum. Compared to the control without diet supplementation, serum Se levels were improved by 10.4% ($P > 0.05$), 27.1% ($P < 0.05$), and 64.6% ($P < 0.01$) with IOSe, Pro-Se2.5, and Pro-Se5.0, respectively. Pro-Se was more favorable to Se absorption and deposition in rats than IOSe, which indicated that the Pro-Se in this study showed a higher bioavailability in mice.

*3.2. Se Accumulation in Liver and Kidney.* The organ masses of liver and kidney were significantly different between Pro-Se and IOSe groups ($P < 0.05$). However, there was no significant difference in organ masses of liver and Kidney between the Pro-Se groups ($P > 0.05$). It is implied that IOSe is toxic to animals, while Pro-Se is essentially nontoxic (Table 2). The Se contents in animal tissues were measured following the studies and the results are shown in Figure 1. The Se content in tissues of Pro-Se2.5 group was 4.21 ppm and 5.56 ppm (liver and kidney) while IOSe groups exhibited elevated levels of Se content, but not to a statistically significant degree. It is implied that Pro-Se could reduce Se accumulation in animal tissues to a certain extent.

Table 2: Effects of Pro-Se and IOSe on organ masses of mice ($n = 10$).

| Mouse group | Liver weights (g) | Kidney weights (g) |
|---|---|---|
| Control group | 1.47 ± 0.05[a] | 0.49 ± 0.04[a] |
| Asthmatic group with saline | 1.30 ± 0.07[b] | 0.37 ± 0.03[b] |
| Asthmatic group with IOSe | 1.31 ± 0.06[b] | 0.34 ± 0.03[b] |
| Asthmatic group with Pro-Se0 | 1.48 ± 0.07[a] | 0.48 ± 0.04[a] |
| Asthmatic group with Pro-Se2.5 | 1.47 ± 0.03[a] | 0.47 ± 0.02[a] |
| Asthmatic group with Pro-Se5.0 | 1.45 ± 0.06[a] | 0.49 ± 0.02[a] |

The different letters in the same column indicate a statistical difference ($P < 0.05$).

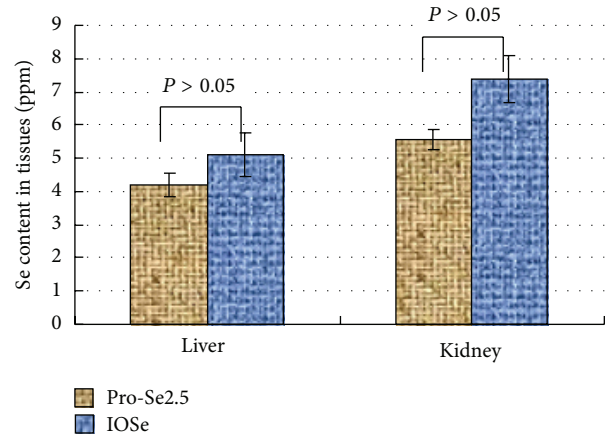


Figure 1: Se contents in animal tissues. The Se content in tissues was not significantly different between Pro-Se and IOSe groups ($P > 0.05$).

*3.3. The Effect of Pro-Se on Oxidative Stress.* In this study, a significant increase in the activity of LPO was observed in asthmatic group, as compared to the control group ($P < 0.001$), whereas Pro-Se2.5 and Pro-Se5.0 treatment significantly ($P < 0.05$, $P < 0.01$, resp.) resulted in decreased LPO levels when compared with asthmatic group (Table 3). Concentrations of GSH were lower in asthmatic group than those in control group (Table 3). Pro-Se produced the increase in the level of GSH. The activity of endogenous antioxidant enzymes was decreased significantly ($P < 0.01$) in the asthmatic group, as compared to the control group, whereas in the Pro-Se2.5 and Pro-Se5.0 groups, Pro-Se treatment showed a significant ($P < 0.05$–$0.01$) restoration in the level of various enzymes as compared with asthmatic group (Table 3). However, there was no significant difference between the asthmatic group and the IOSe treated group (Table 3).

*3.4. The Effect of Pro-Se on Inflammatory Mediators.* Figure 2 shows that ovalbumin-induced asthma significantly

Table 3: Effect of Pro-Se and IOSe on GSH and LPO levels and the activity of various enzymes.

| Different groups | GSH (nmol/mL) | LPO (nmol/mL) | GPx | GR | GST | CAT | $Na^+K^+$ ATPase |
|---|---|---|---|---|---|---|---|
| Control group | $1.830 \pm 0.011^*$ | $14.33 \pm 0.56^{***}$ | $16.00 \pm 2.23^{**}$ | $36.56 \pm 2.56^{**}$ | $17.44 \pm 1.23^{**}$ | $7.22 \pm 0.32^{**}$ | $4.56 \pm 0.60^*$ |
| Asthmatic group with saline | $1.112 \pm 0.011$ | $20.01 \pm 1.41$ | $7.89 \pm 0.33$ | $21.11 \pm 2.23$ | $9.07 \pm 1.11$ | $4.66 \pm 0.10$ | $2.22 \pm 0.20$ |
| Asthmatic group with IOSe | $1.401 \pm 0.022^*$ | $19.82 \pm 1.22$ | $8.10 \pm 0.32$ | $24.31 \pm 2.02$ | $10.60 \pm 0.66$ | $4.88 \pm 0.32$ | $3.00 \pm 0.31$ |
| Asthmatic group with Pro-Se0 | $1.300 \pm 0.011^*$ | $17.80 \pm 1.11$ | $6.12 \pm 0.30$ | $20.31 \pm 2.00$ | $9.60 \pm 0.33$ | $3.80 \pm 0.25$ | $2.00 \pm 0.30$ |
| Asthmatic group with Pro-Se2.5 | $1.500 \pm 0.021^*$ | $17.23 \pm 2.00^*$ | $13.23 \pm 1.30^*$ | $27.50 \pm 2.11^{**}$ | $15.50 \pm 1.15^*$ | $5.45 \pm 0.55^*$ | $4.32 \pm 0.30^*$ |
| Asthmatic group with Pro-Se5.0 | $1.500 \pm 0.033^*$ | $14.33 \pm 3.21^*$ | $14.11 \pm 1.12^{**}$ | $30.21 \pm 6.03^{**}$ | $17.66 \pm 2.33^{**}$ | $6.66 \pm 0.44^*$ | $4.11 \pm 0.21^*$ |

Values are shown as means ± SEM. $^*P < 0.05$ versus asthmatic group, $^{**}P < 0.01$ versus asthmatic group, and $^{***}P < 0.001$ versus asthmatic group.

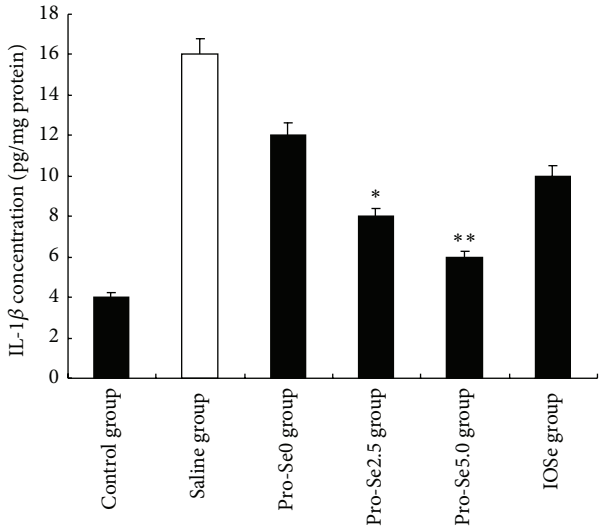


Figure 2: Effect of Pro-Se and IOSe on IL-1$\beta$ concentration. Values are shown as means ± SEM. $^*P < 0.05$ versus asthmatic group.

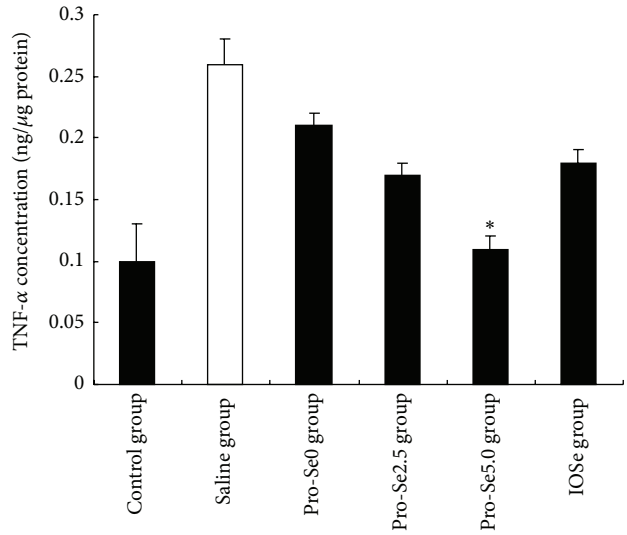


Figure 3: Effect of Pro-Se and IOSe on TNF-$\alpha$ concentration. Values are shown as means ± SEM. $^*P < 0.05$ versus asthmatic group.

increased protein concentration of IL-1$\beta$ in the blood. Pro-Se2.5 and Pro-Se5.0 treatment decreased the level of IL-1$\beta$ as compared to the asthmatic group, respectively ($P < 0.01$, $P < 0.05$). As shown in Figure 3, the levels of TNF-$\alpha$ elevated significantly after ovalbumin-induced asthma. Pro-Se5.0 suppressed this response ($P < 0.05$). However the same result did not occur in the IOSe treated group.

*3.5. The Effect of Pro-Se on Activation of NF-κB.* As shown in Figure 4, asthma significantly induced activated NF-$\kappa$B above control levels, and as hypothesized, Pro-Se2.5 and Pro-Se5.0 significantly suppressed this response. However, the same results did not occur in the Pro-Se0 treated group and IOSe treated group.

## 4. Discussion

It has been reported that selenium supplementation might be beneficial to patients with intrinsic asthma [13]. However, the less absorbed and bioavailability limits its role as a therapeutic agent for asthma [10–12]. In the present study, we isolated Pro-Se from *Ganoderma lucidum* and compared the difference of the antioxidant and anti-inflammatory activities between organic selenium (Pro-Se) and inorganic selenium (IOSe) in the model of asthma.

In this study, we have demonstrated a correlation between Se and asthma. Asthma led to a decrease in serum Se level. In our *in vivo* study, we have presented evidence for a significant increase in serum Se level in Pro-Se treated asthmatic mice while IOSe did not exhibit any such effects. It indicates Pro-Se may improve the bioavailability of Se in mice. At the same time, the Se content in tissues of Pro-Se2.5 group was lower than those of IOSe groups. It is implied that Pro-Se could reduce Se accumulation in animal tissues to a certain extent.

The lungs of asthmatic patients are exposed to oxidative stress due to the generation of reactive oxygen as a consequence of chronic airway inflammation. The available evidence tends to support the concept that the oxidant/antioxidant equilibrium is disturbed in asthma [14,

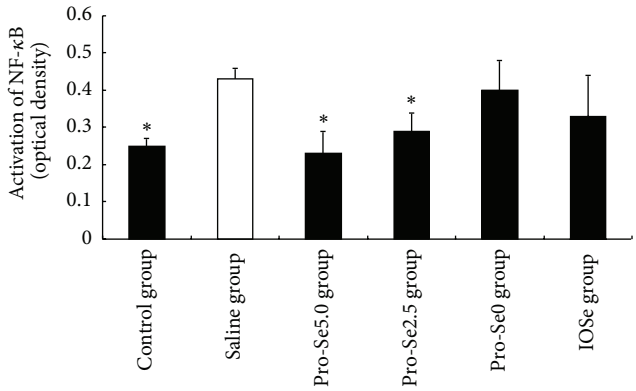


Figure 4: Effect of Pro-Se and IOSe on activation of NF-$\kappa$B. Values are shown as means ± SEM. $^{*}P < 0.05$ versus asthmatic group.

15]. Therefore, the measurement of endogenous antioxidants enzymes, that is, GPx, GR, CAT, and GST as well as $Na^{+}K^{+}$ATPase, has been performed to estimate the amount of oxidative stress. Se plays an important role in the antioxidant defence system [16, 17]. In the present study, asthmatic mice had lower levels of antioxidant enzymes such as GST, GPx, CAT, GR, and catalase activities, whereas in the Se group, Pro-Se treatment showed a significant restoration in the level of various enzymes as compared with asthmatic group. The same results did occur in the IOSe group. These findings are consistent with the presented evidence for a significant increase in serum Se level in Pro-Se treated asthmatic mice.

Adequate assessment of inflammatory cells, cytokines, chemokines, and anti-inflammatory molecules is essential for understanding, monitoring, and treating lung diseases. Among these inflammatory mediators, IL-1$\beta$ and TNF-$\alpha$ are of particular importance because they play a major role in coordinating mechanisms that command proinflammation. The suppression of these proinflammatory mediators has been found to reduce the severity of the inflammatory reaction [18]. We found that Pro-Se significantly reduced the levels of IL-1$\beta$ and TNF-$\alpha$. However, the same result did not occur in the IOSe treated group. These studies support our hypothesis that Pro-Se may enhance Se anti-inflammatory effects.

NF-$\kappa$B activation is correlated with significant increases in IL-1$\beta$ and TNF-$\alpha$ mRNA levels [19]. Therefore, we hypothesized that Pro-Se may potentially show beneficial effects by decreasing the expression of NF-$\kappa$B. As shown in Figure 4, NF-$\kappa$B expression in the saline group was significantly higher than that in the control group ($P < 0.01$), and Pro-Se treatment significantly suppressed asthma-induced NF-$\kappa$B expression. These results suggest that the inhibitory effects of Pro-Se on expression of the NF-$\kappa$B p50 subunit are associated with increase in serum Se level in Pro-Se treated asthmatic mice.

In conclusion, this study clearly shows that organic selenium (Pro-Se) is able to counteract the asthma in an experimental model by using a variety of testing systems. Also, our experiments have demonstrated profound differences between the activities of organic selenium and inorganic selenium in experimental conditions. These data provide an important proof of the concept that organic selenium might be a new potential therapy for the management of asthma in humans. The potential application of organic selenium needs to be further studied.

## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

## References

[1] D. K. Agrawal and Z. Shao, "Pathogenesis of allergic airway inflammation," *Current Allergy and Asthma Reports*, vol. 10, no. 1, pp. 39–48, 2010.

[2] H. S. McGee, A. L. Stallworth, T. Agrawal, Z. Shao, L. Lorence, and D. K. Agrawal, "Fms-like tyrosine kinase 3 ligand decreases T helper type 17 cells and suppressors of cytokine signaling proteins in the lung of house dust mite-sensitized and -challenged mice," *American Journal of Respiratory Cell and Molecular Biology*, vol. 43, no. 5, pp. 520–529, 2010.

[3] F. E. Hargreave and P. Nair, "The definition and diagnosis of Asthma," *Clinical and Experimental Allergy*, vol. 39, no. 11, pp. 1652–1658, 2009.

[4] J. Chakir, Q. Hamid, M. Bossé, L.-P. Boulet, and M. Laviolette, "Bronchial inflammation in corticosteroid-sensitive and corticosteroid-resistant asthma at baseline and on oral corticosteroid treatment," *Clinical and Experimental Allergy*, vol. 32, no. 4, pp. 578–582, 2002.

[5] Y. Sumi and Q. Hamid, "Airway remodeling in asthma," *Allergology International*, vol. 56, no. 4, pp. 341–348, 2007.

[6] J. Stone, L. J. Hinks, R. Beasley, S. T. Holgate, and B. A. Clayton, "Reduced selenium status of patients with asthma," *Clinical Science*, vol. 77, no. 5, pp. 495–500, 1989.

[7] A. Flatt, N. Pearce, C. D. Thomson, M. R. Sears, M. F. Robinson, and R. Beasley, "Reduced selenium in asthmatic subjects in New Zealand," *Thorax*, vol. 45, no. 2, pp. 95–99, 1990.

[8] D. C. Mahan and Y. Y. Kim, "Effect of inorganic or organic selenium at two dietary levels on reproductive performance and tissue selenium concentrations in first-parity gilts and their progeny," *Journal of Animal Science*, vol. 74, no. 11, pp. 2711–2718, 1996.

[9] D. C. Mahan and N. A. Parrett, "Evaluating the efficacy of selenium-enriched yeast and sodium selenite on tissue selenium retention and serum glutathione peroxidase activity in grower and finisher swine," *Journal of Animal Science*, vol. 74, no. 12, pp. 2967–2974, 1996.

[10] Z. Gajčević, G. Kralik, E. Has-Schön, and V. Pavić, "Effects of organic selenium supplemented to layer diet on table egg freshness and selenium content," *Italian Journal of Animal Science*, vol. 9, no. 2, pp. 189–199, 2009.

[11] F. Z. KüÜkbay, H. Yazlak, I. Karaca et al., "The effects of dietary organic or inorganic selenium in rainbow trout (*Oncorhynchus mykiss*) under crowding conditions," *Aquaculture Nutrition*, vol. 15, no. 6, pp. 569–576, 2009.

[12] J. B. Taylor, J. W. Finley, and J. S. Caton, "Effect of the chemical form of supranutritional selenium on selenium load and selenoprotein activities in virgin, pregnant, and lactating rats," *Journal of Animal Science*, vol. 83, no. 2, pp. 422–429, 2005.

[13] J. Kadrabova, A. Mad'aric, Z. Kovacikova, F. Podivinsky, E. Ginter, and F. Gazdik, "Selenium status is decreased in patients with intrinsic asthma," *Biological Trace Elemement Research*, vol. 52, no. 3, pp. 241–248, 1996.

[14] A. A. Andreadis, S. L. Hazen, S. A. A. Comhair, and S. C. Erzurum, "Oxidative and nitrosative events in asthma," *Free Radical Biology and Medicine*, vol. 35, no. 3, pp. 213–225, 2003.

[15] P. A. J. Henricks and F. P. Nijkamp, "Reactive oxygen species as mediators in asthma," *Pulmonary Pharmacology and Therapeutics*, vol. 14, no. 6, pp. 409–421, 2001.

[16] L. Hasselmark, R. Malmgren, O. Zetterstrom, and G. Unge, "Selenium supplementation in intrinsic asthma," *Allergy*, vol. 48, no. 1, pp. 30–36, 1993.

[17] F. Gazdik, J. Kadrabova, and K. Gazdikova, "Decreased consumption of corticosteroids after selenium supplementation in corticoid-dependent asthmatics," *Bratislavske Lekarske Listy*, vol. 103, no. 1, pp. 22–25, 2002.

[18] J. L. Stow, P. Ching Low, C. Offenhäuser, and D. Sangermani, "Cytokine secretion in macrophages and other cells: pathways and mediators," *Immunobiology*, vol. 214, no. 7, pp. 601–612, 2009.

[19] A. Ugusman, Z. Zakaria, C. Kien Hui, and N. A. Megat Mohd Nordin, "*Piper sarmentosum* inhibits ICAM-1 and Nox4 gene expression in oxidative stress-induced human umbilical vein endothelial cells," *BMC Complementary and Alternative Medicine*, vol. 11, article 31, 2011.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2014, Article ID 679259, 6 pages
http://dx.doi.org/10.1155/2014/679259

*Research Article*

# Protective Effect of *Agaricus brasiliensis* on STZ-Induced Diabetic Neuropathic Pain in Rats

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Received 16 October 2013; Accepted 12 November 2013; Published 12 January 2014

Academic Editor: Bo Cui



*Objective*. The present investigation examined the neuroprotective effect of *Agaricus brasiliensis* (AbS) against STZ-induced diabetic neuropathic pain in laboratory rats. STZ-induced diabetic rats were administered orally with AbS. Body weight, serum glucose, and behavioral parameters were measured before and at the end of the experiment to see the effect of AbS on these parameters. After 6 weeks of treatments, all animals were sacrificed to study various biochemical parameters. Treatment with AbS 80 mg/kg in diabetic animals showed significant increase in body weight, pain threshold, and paw withdrawal threshold and significant decrease in serum glucose, LPO and NO level, Na-K-ATPase level, and TNF-$\alpha$ and IL-1$\beta$ level as compared to vehicle treated diabetic animals in dose and time dependent manner. AbS can offer pain relief in PDN. This may be of potential benefit in clinical practice for the management of diabetic neuropathy.

## 1. Introduction

Diabetic neuropathy is the most common of secondary complications associated with diabetes mellitus and is characterized by the slowing of nerve conduction velocity, elevated pain, sensory loss, and nerve fiber degeneration. Diabetes induced neuropathic pain is recognized as one of the most difficult types of pain to treat with conventional analgesics.

Current treatment of peripheral diabetic neuropathy (PDN) involves the use of tricyclic antidepressant, selective serotonin reuptake inhibitors [1], anticonvulsants, opioids and antioxidant protein kinase C inhibitors, COX-2 inhibitors [2], nonsteroidal anti-inflammatory drugs as mild analgesics, and so on. However, these therapies provide relief only to a fraction of patients and their side effect profiles limit their use [3, 4]. Thus, there is a need for new therapeutic interventions targeting primary mechanisms resulting in nerve damage in PDN. Recently, from the point of view of "self-medication" or "preventive medicine," several dietary supplements are used in the prevention of lifestyle related diseases including diabetes.

Mushrooms and primarily basidiomycetous fungi are valuable foods that are low in calories and high in minerals, essential amino acids, vitamins, and fibers [5]. Some of them produce substances with potential medical effect and are called medicinal mushrooms [6]. The basidiomycete *Agaricus brasiliensis* (AbS) is native to Brazil and is widely grown in Japan because of its medicinal properties. It is widely used for nonprescript, medicinal purposes, both as an edible mushroom and in the form of extracts. AbS has traditionally been used for the prevention of a range of diseases, including cancer, hepatitis, atherosclerosis, hypercholesterolemia, diabetes, and dermatitis [7–9].

However, the role of AbS in diabetic complications has not been investigated. The aim of the present investigation was to evaluate the neuroprotective effect of AbS against STZ-induced diabetic neuropathic pain in laboratory rats by assessing various behavioral and biochemical parameters.

## 2. Material and Methods

*2.1. Animals.* Healthy male adult Wistar rats (2 months old and weighing 225 ± 25 g) were used in the study. This study was performed in accordance with the Guide for the Care and Use of Laboratory Animals. Care was taken to minimize discomfort, distress, and pain to the animals.

*2.2. Chemicals.* Streptozotocin was purchased from Sigma (USA) and was dissolved in 0.1 N citrate buffers.

*2.3. Fermented Mushroom of AbS Extract.* The fermented mushroom of AbS was produced by the way to produce *Coprinus comatus* [10]. The aqueous extraction was performed by adding 100 mL boiling water to 10 g air-dried mycelium. The infusion stood at room temperature for 30 minutes. After cooling and filtration, the extract was frozen and concentrated by lyophilization for five days overnight, in order to obtain the AbS (0.68 g).

*2.4. Induction and Assessment of Diabetes in Rats.* Experimental diabetes was induced by a single intraperitoneal (i.p.) injection of STZ (50 mg kg$^{-1}$) freshly dissolved in citrate buffer pH. Serum glucose level was assessed by using enzymatic glucose oxidase peroxidase commercially available kit method, 72 h after STZ induction. Only rats with blood glucose concentration more than 240 mg/dL were considered diabetic and used for the study. Body weight and serum glucose were measured before and at the end of the experiment to see the effect of pharmacological interventions on these parameters. The body weights of the mice were measured every two weeks.

*2.5. Treatment Schedule and Experimental Protocol.* Forty hyperglycemic rats were selected and allocated equally into 4 groups. From then on, the 4 groups of hyperglycemic rats were administered orally (gavage) with saline, AbS (20 mg/kg/d), AbS (40 mg/kg/d), and AbS (80 mg/kg/d), respectively. AbS was dissolved in the same amount of saline. The other 10 normal rats were administered orally with the saline and used as the control group.

Body weight of all animals was recorded on the 0, 2nd, 4th, and 6th weeks of treatment. Blood of all animals was collected through retro-orbital route initially and on the 6th week of treatment to measure the serum glucose levels.

*2.6. Behavioral Tests.* Development of neuropathy was assessed in control and diabetic animals from all groups by evaluation of pain thresholds on the 0, 2nd, 4th, and 6th weeks of respective treatment by assessment of thermal/mechanical hyperalgesia and thermal allodynia [11–13].

*2.7. Biochemical Assessment.* All animals were sacrificed at the end of the study; that is, 6th week and sciatic nerves were rapidly removed and weighed. Tissue homogenates were prepared with 0.1 M Tris-HCl buffer (pH7.4) and supernatant of homogenates was employed to estimate superoxide dismutase (SOD) [14], lipid peroxidation (LPO) [15], nitric oxide (NO content) [16], and membrane bound inorganic phosphate (Na+K+ATPase) [17]. TNF-$\alpha$ and IL-1$\beta$ concentrations were quantified by ELISA (Neobioscience, China). According to the manufacturer's instructions, the absorbance (A) was detected at 450 nm (A450). The content of each sample was obtained according to the standard curve.

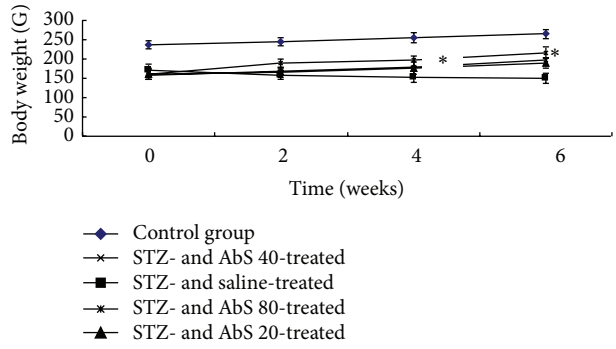


Figure 1: Effect of AbS treatment on body weight. Results are expressed as mean ± SEM ($n = 6$). The data was analysed using and one-way analysis of variance (ANOVA) followed by Dunnett's test. $^{*}P < 0.05$ versus diabetic group.

*2.8. Acute Toxicity Study.* The AbS was administered at a dose of 2000 mg/kg orally to ten healthy adult female Wistar rats. Animals were observed individually for the first four hours after dosing for the presence of any clinical signs, such as changes in skin fur, lacrimation, salivation, piloerection, diarrhea, and mortality. The gross behaviors were observed. Surviving animals were observed for outcomes for a period of 24 hours. The animals were kept under supervision up to 14 days for any sign of toxicity or mortality (OECD Guideline, 2000).

*2.9. Statistical Analysis.* All data were analyzed by a one-way analysis of variance, and the differences between means were established by Duncan's multiple-range test. The data are shown as the mean ± SEM. The significant level of 5% ($P < 0.05$) was used as the minimum acceptable probability for the difference between the means.

## 3. Results

*3.1. Effect of AbS on Body Weight and Blood Glucose Levels.* The STZ-treated animals had significantly reduced body weight than the control rats. The average blood glucose level of the STZ-treated animals was significantly higher as compared to the control animals. Treatment with AbS 80 mg/kg in diabetic animals showed significant increase in body weight (Figure 1) and significant decrease in serum glucose (Table 1) as compared to vehicle treated diabetic animals in dose and time dependent manner.

*3.2. Effect of AbS Treatment on Thermal Hyperalgesia.* The nociceptive threshold was significantly lower in diabetic rats as compared with control animals tested in the tail immersion (Figure 2). Treatment of diabetic rats with AbS 80 mg/kg

Table 1: Effect of AbS and other treatments on blood glucose levels in STZ-hyperglycemic rats.

| Different groups | Blood glucose (mg/dL) |
|---|---|
| STZ- and saline-treated | 310.20 ± 2.111 |
| STZ- and AbS 80-treated | 140.56 ± 2.150** |
| STZ- and AbS 40-treated | 196.60 ± 3.226* |
| STZ- and AbS 20-treated | 238.93 ± 2.836 |
| Control group | 105.94 ± 1.223 |

Values are shown as means ± SEM. *$P < 0.05$ versus diabetic group, **$P < 0.01$ versus diabetic group.

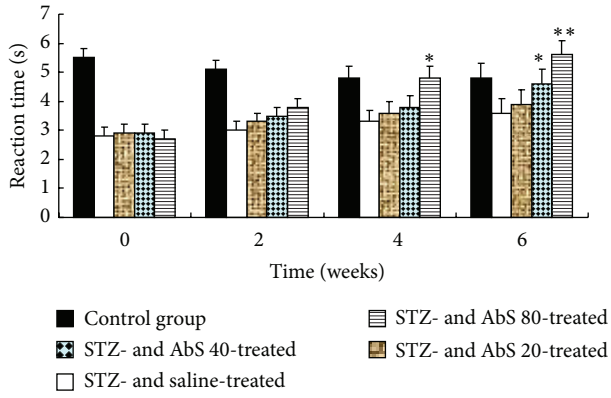


Figure 2: Effect of AbS treatment on tail withdrawal latency. Results are expressed as Mean ± SEM ($n = 6$). The data was analysed using and one-way analysis of variance (ANOVA) followed by Dunnett's test. *$P < 0.05$ versus diabetic group, **$P < 0.01$ versus diabetic group.

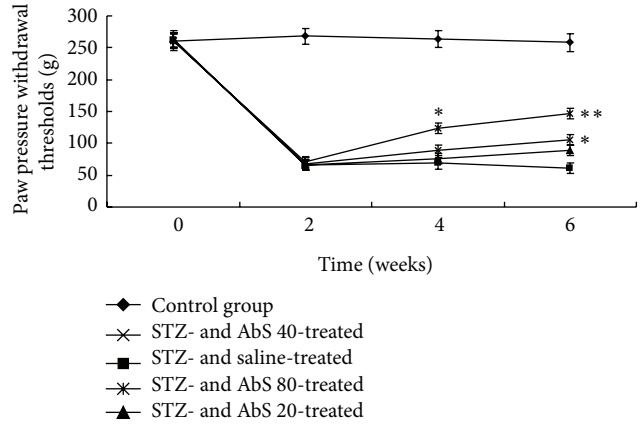


Figure 3: Effect of AbS treatment on paw withdrawal threshold. Results are expressed as mean ± SEM ($n = 6$). The data was analysed using and one-way analysis of variance (ANOVA) followed by Dunnett's test. *$P < 0.05$ versus diabetic group, **$P < 0.01$ versus diabetic group.

induced a statistically significant increase in pain threshold after four weeks of treatment, which was further increased after six weeks of treatment in dose dependant manner. The AbS 40 mg/kg treatment showed significant increase in pain threshold level as compared to diabetic control only after six weeks of treatment.

*3.3. Effect of AbS Treatment on Mechanical Hyperalgesia.* There was no significant change in the mean paw withdrawal threshold of control rats during the time period of 6 weeks. A significant decrease in ($P < 0.05$) mean paw withdrawal threshold was produced in the diabetic rats (66.21 ± 6.40 g) after 2 weeks of STZ injection as compared to control animals (268.11 ± 14.90 g). In rats receiving the treatment of AbS (40 and 80 mg/kg), mean paw withdrawal threshold was significantly and dose dependently increased (105.50 ± 11.00 and 146.23 ± 22.00 g resp.) compared to diabetic control rats (Figure 3).

*3.4. Effect of AbS Treatment on Thermal Allodynia.* Marked thermal allodynia was observed in the diabetic animals as evidenced by a reduction in the pain thresholds compared to control animals (Figure 4). Treatment of diabetic rats with AbS induced a significant increase in pain threshold compared to diabetic animals after six weeks of treatment in dose dependant manner.

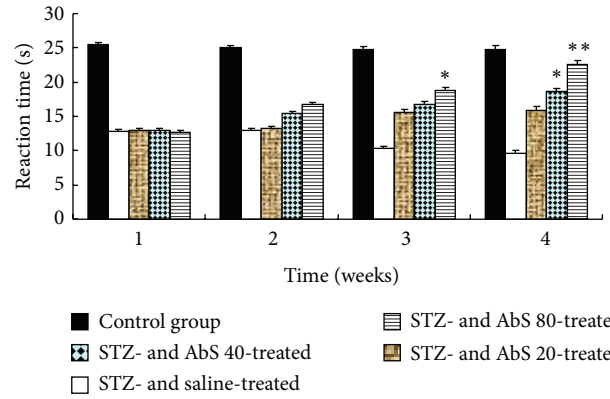


Figure 4: Effect of AbS treatment on paw withdrawal latency in warm plate test. Results are expressed as mean ± SEM ($n = 6$). The data was analysed using and one-way analysis of variance (ANOVA) followed by Dunnett's test. *$P < 0.05$ versus diabetic group, **$P < 0.01$ versus diabetic group.

*3.5. Effect of AbS Treatment Lipid Peroxide Profile and Nitrosative Stress.* After 6 weeks of STZ injection, LPO and NO level in diabetic rats was significantly increased as compared to control rats. The LPO level in AbS 80-treated rats was decreased (6.11 ± 0.40 nM/mg of protein, $P < 0.01$) significantly and dose dependently compared to diabetic rats. At the same time, the NO level in AbS 80-treated rats

Table 2: Effect of AbS and other treatments on LPO and NO level.

| Different groups | LPO (nM/mg of protein) | NO ($\mu$g/mL) |
|---|---|---|
| STZ- and saline-treated | 12.29 ± 0.33 | 288.30 ± 6.25 |
| STZ- and AbS 80-treated | 6.11 ± 0.40** | 169.10 ± 11.21* |
| STZ- and AbS 40-treated | 8.22 ± 0.32* | 223.21 ± 22.71 |
| STZ- and AbS 20-treated | 10.00 ± 0.12 | 242.20 ± 18.70 |
| Control group | 2.20 ± 0.21 | 108.22 ± 8.13 |

Values are shown as means ± SEM. * $P < 0.05$ versus diabetic group, ** $P < 0.01$ versus diabetic group.

Table 3: Effect of AbS and other treatments on SOD and Na+K+ATPase level.

| Different groups | SOD (U/mg of protein) | Na+K+ATPase ($\mu$mol/mg of protein) |
|---|---|---|
| STZ- and saline-treated | 5.80 ± 0.66 | 2.29 ± 0.30 |
| STZ- and AbS 80-treated | 20.80 ± 0.63* | 9.11 ± 0.40* |
| STZ- and AbS 40-treated | 11.60 ± 0.22 | 6.22 ± 0.32 |
| STZ- and AbS 20-treated | 8.93 ± 0.36 | 4.00 ± 0.10 |
| Control group | 29.66 ± 0.80 | 12.20 ± 1.20 |

Values are shown as means ± SEM. * $P < 0.05$ versus diabetic group.

was significantly and dose dependently decreased (169.10 ± 11.21 $\mu$g/mL, $P < 0.05$) as compared to diabetic rats (Table 2).

*3.6. Effect of AbS Treatment on Superoxide Dismutase Profile and Membrane Bound Inorganic Phosphate.* Intraperitoneal administration of STZ resulted in significant decrease ($P < 0.05$) in SOD and Na-K-ATPase level in diabetic rats compared to control rats. SOD level in AbS-80 treated rats was increased (20.80 ± 0.63 U/mg of protein, $P < 0.05$) significantly and dose dependently as compared to diabetic rats. At the same time, the Na-K-ATPase level in AbS 80-treated rats was significantly and dose dependently pendently increased (9.11 ± 0.40 $\mu$mol/mg of protein, $P < 0.05$) as compared to diabetic rats (Table 3).

*3.7. Effect of AbS Treatment on TNF-$\alpha$ and IL-1$\beta$ Level.* As shown in Table 4, the levels of TNF-$\alpha$ and IL-1$\beta$ were significantly increased after STZ injection. AbS 80 suppressed STZ-induced TNF-$\alpha$ and IL-1$\beta$ production ($P < 0.05$). Moreover, the TNF-$\alpha$ and IL-1$\beta$ level in AbS treated rats was dose dependently decreased.

*3.8. Toxicity Profile.* All the animals did not show any sign of toxicity or mortality in the first four hours after dosing and thereafter up to the next 14 days.

## 4. Discussion

Diabetic neuropathy is characterized by clinical features like allodynia and hyperalgesia due to elevated nociceptive response. Similar symptoms are exhibited by STZ-induced diabetic animals [18]. STZ is an antibiotic extracted from *Streptomyces acromogenes* and is diabetogenic due to a selective cytotoxic action upon pancreatic $\beta$ cell [19]. STZ injected rats exhibit clinicopathological features including biochemical, oxidative, and metabolic changes which also presented in humans [20].

In the present investigation, loss in body weight was halted in AbS treated animals when compared with STZ-induced diabetic animals. It indicates that the improvement on body weight may be attributed to the improvement on metabolic dysfunction in diabetic rats. The hypoglycemic effect of AbS was significant exhibiting observation similar to that in earlier reports [21].

It has been reported earlier that STZ-induced diabetic neuropathic pain is characterized by hyperalgesia and allodynia [22, 23] and was also found in the present study following STZ injection. A decrease in pain threshold was observed in STZ-diabetic rats using a mildly noxious stimulus such as mechanical force. Moreover, there was also a similar increase in thermal hyperalgesic activity in diabetic rats as compared with normal rats when subjected to thermal stimuli. In the present investigation, we found that administration of AbS dose dependently reversed STZ-induced thermal hyperalgesia and mechanical allodynia. However, the mechanism of antihyperalgesic and antiallodynic effect of AbS on STZ-induced PDN needs to be further studied.

In diabetes, pain threshold of the neurons is reduced due to oxidative stress generated by free radicals such as super oxide dismutase, hydroxyl radical, and peroxynitrite which impair blood supply to the neurons leading to impaired neuronal function and hypoxia [24, 25]. These conditions were investigated by assessing the biochemical markers like SOD, LPO, Na-K-ATPase, and NO.

We observed a significant increase in LPO and reduction in endogenous antioxidant enzymes like SOD and Na-K-ATPase activity in sciatic nerves of diabetic rats. Treatment with AbS for six weeks restored above mentioned biochemical parameters in diabetic rats in dose dependent manner.

Nitric oxide (NO) is an unconventional intracellular messenger playing a vital role in various pathological and physiological processes. A localized increase in NO level leads

Table 4: Effect of AbS and other treatments on TNF-$\alpha$ level.

| Different groups | TNF-$\alpha$ (pg/mL) | IL-1$\beta$ (pg/mL) |
|---|---|---|
| STZ- and saline-treated | 128.29 ± 10.33 | 130.29 ± 22.30 |
| STZ- and AbS 80-treated | 76.11 ± 10.40** | 56.55 ± 9.22** |
| STZ- and AbS 40-treated | 108.22 ± 10.30* | 94.11 ± 8.40* |
| STZ- and AbS 20-treated | 111.0 0 ± 10.10 | 116.10 ± 22.41** |
| Control group | 52.23 ± 4. 20 | 22.20 ± 2. 20 |

Values are shown as means ± SEM. * $P < 0.05$ versus diabetic group, ** $P < 0.01$ versus diabetic group.

to the formation of peroxynitrite by reacting with superoxide anions which causes rapid protein nitration or nitrosylation, lipid peroxidation, DNA damage, and cell death and which intern contribute to elevated pain [26]. NO, an indicator of nitrosative stress, was measured and found to be increased in the STZ-diabetic rats. We observed that AbS treatments dose dependently attenuated nitrite level in STZ-diabetic rats that exhibited thermal hyperanalgaesia and mechanical allodynia.

Evidence from both animal models and humans indicates that systemic inflammation is involved in the pathophysiological processes of diabetes [27]. Moreover, TNF-$\alpha$ has been reported to initiate the release of other inflammatory cytokines including IL-1$\beta$ and IL-2 that are responsible for causing neuropathic pain [28, 29]. Uses of agents that suppress cytokine elevation have been advocated to be used to treat diabetic complication. The present investigation demonstrates that AbS was able to dose dependently reduce the population of cytokine. Therefore, AbS may exhibit its neuroprotective effect by downregulation of cytokine including TNF-$\alpha$ and IL-1$\beta$, the important mediators of neuropathic pain.

## 5. Conclusion

These results suggest that AbS attenuated STZ-induced neuropathic pain behaviours by inhibiting oxidative, nitrosative stress, cytokines activation, and hypoglycemic effect of Abs. This may be of potential benefit in clinical practice for the management of diabetic neuropathy.

## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

## Acknowledgments

This study was supported by the National Natural Science Foundation of China (Grant nos. 81102608 and 31170992) and the Zhejiang Science and Technology Key Innovation Team of Integrative Osteoarthropathy.

## References

[1] K. McKeage, "Treatment options for the management of diabetic painful neuropathy: best current evidence," *Current Opinion in Neurology*, vol. 20, no. 5, pp. 553–557, 2007.

[2] A. P. Kellogg, H. T. Cheng, and R. Pop-Busui, "Cyclooxygenase-2 pathway as a potential therapeutic target in diabetic peripheral neuropathy," *Current Drug Targets*, vol. 9, no. 1, pp. 68–76, 2008.

[3] M. S. Chong and J. Hester, "Diabetic painful neuropathy: current and future treatment options," *Drugs*, vol. 67, no. 4, pp. 569–585, 2007.

[4] A. B. O'Connor, "Neuropathic pain: quality-of-life impact, costs and cost effectiveness of therapy," *PharmacoEconomics*, vol. 27, no. 2, pp. 95–112, 2009.

[5] F. Firenzuoli, L. Gori, and G. Lombardo, "The medicinal mushroom Agaricus blazei murrill: review of literature and pharmaco-toxicological problems," *Evidence-Based Complementary and Alternative Medicine*, vol. 5, no. 1, pp. 3–15, 2008.

[6] M. A. A. Al-Fatimi, W.-D. Jülich, R. Jansen, and U. Lindequist, "Bioactive components of the traditionally used mushroom Podaxis pistillaris," *Evidence-Based Complementary and Alternative Medicine*, vol. 3, no. 1, pp. 87–92, 2006.

[7] S. P. Wasser and A. L. Weis, "Therapeutic effects of substances occurring in higher basidiomycetes mushrooms: a modern perspective," *Critical Reviews in Immunology*, vol. 19, no. 1, pp. 65–96, 1999.

[8] C. H. Hsu, Y. L. Liao, S. C. Lin, K. Hwang, and P. Chou, "The mushroom *Agaricus brasiliensis* in combination with metformin and gliclazide improves insulin resistance in type 2 diabetes: a randomized, double-blinded, and placebo-controlled clinical trial," *Journal of Alternative and Complementary Medicine*, vol. 13, no. 1, pp. 97–102, 2007.

[9] L. Chen, H. J. Shao, and Y. B. Su, "Coimmunization of *Agaricus brasiliensis* extract with hepatitis B virus core protein through DNA vaccine enhances cellular and humoral immune responses," *International Immunopharmacology*, vol. 4, no. 3, pp. 403–409, 2004.

[10] C. Han, J. Yuan, Y. Wang, and L. Li, "Hypoglycemic activity of fermented mushroom of Coprinus comatus rich in vanadium," *Journal of Trace Elements in Medicine and Biology*, vol. 20, no. 3, pp. 191–196, 2006.

[11] L. O. Randall and J. Selitto, "A method for measurement of analgesic activity of inflamed tissue," *Archives Internationales de Pharmacodynamie et de Thérapie*, vol. 111, no. 4, pp. 209–219, 1957.

[12] S. R. Chaplan, F. W. Bach, J. W. Pogrel, J. M. Chung, and T. L. Yaksh, "Quantitative assessment of tactile allodynia in the rat paw," *Journal of Neuroscience Methods*, vol. 53, no. 1, pp. 55–63, 1994.

[13] R. Necker and R. F. Hellon, "Noxious thermal input from the rat tail: modulation by descending inhibitory influences," *Pain*, vol. 4, no. 3, pp. 231–242, 1978.

[14] H. P. Misra and I. Fridovich, "The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide

dismutase," *Journal of Biological Chemistry*, vol. 247, no. 10, pp. 3170–3175, 1972.

[15] T. F. Slater and B. C. Sawyer, "The stimulatory effects of carbon tetrachloride and other halogenoalkanes on peroxidative reactions in rat liver fractions in vitro. General features of the systems used," *Biochemical Journal*, vol. 123, no. 5, pp. 805–814, 1971.

[16] K. M. Miranda, M. G. Espey, and D. A. Wink, "A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite," *Nitric Oxide*, vol. 5, no. 1, pp. 62–71, 2001.

[17] S. L. Bonting, "Membrane and ion transport," in *Presence of Enzyme Systems in Mammalian Tissues*, E. E. Bilter, Ed., vol. 2, pp. 257–263, Wiley Interscience, London, UK, 1970.

[18] N. A. Calcutt and S. R. Chaplan, "Spinal pharmacology of tactile allodynia in diabetic rats," *British Journal of Pharmacology*, vol. 122, no. 7, pp. 1478–1482, 1997.

[19] N. Rakieten, M. L. Rakieten, and M. R. Nadkarni, "Studies on the diabetogenic action of streptozotocin (NSC-37917)," *Cancer Chemotherapy Reports*, vol. 29, pp. 91–98, 1963.

[20] C. Courteix, A. Eschalier, and J. Lavarenne, "Streptozocin-induced diabetic rats: behavioural evidence for a model of chronic pain," *Pain*, vol. 53, no. 1, pp. 81–88, 1993.

[21] C. H. Hsu, Y. L. Liao, S. C. Lin, K. Hwang, and P. Chou, "The mushroom *Agaricus brasiliensis* in combination with metformin and gliclazide improves insulin resistance in type 2 diabetes: a randomized, double-blinded, and placebo-controlled clinical trial," *Journal of Alternative and Complementary Medicine*, vol. 13, no. 1, pp. 97–102, 2007.

[22] M. Meeus and J. Nijs, "Central sensitization: a biopsychosocial explanation for chronic widespread pain in patients with fibromyalgia and chronic fatigue syndrome," *Clinical Rheumatology*, vol. 26, no. 4, pp. 465–473, 2007.

[23] K. T. Velazques, H. Mohammad, and S. M. Swetzer, "Protein kinase in pain: involvement of multiple isoforms," *Pharmacological Research*, vol. 55, no. 6, pp. 578–589, 2007.

[24] M. Friederich, P. Hansell, and F. Palm, "Diabetes, oxidative stress, nitric oxide and mitochondria function," *Current Diabetes Reviews*, vol. 5, no. 2, pp. 120–144, 2009.

[25] A. Ozkul, M. Ayhan, C. Yenisey, A. Akyol, E. Guney, and F. A. Ergin, "The role of oxidative stress and endothelial injury in diabetic neuropathy and neuropathic pain," *Neuroendocrinology Letters*, vol. 31, no. 2, pp. 261–264, 2010.

[26] S. Y. Kim, J. H. Lee, E. S. Yang, I. S. Kil, and J. Park, "Human sensitive to apoptosis gene protein inhibits peroxynitrite-induced DNA damage," *Biochemical and Biophysical Research Communications*, vol. 301, no. 3, pp. 671–674, 2003.

[27] M. R. Dasu, S. Ramirez, and R. R. Isseroff, "Toll-like receptors and diabetes: a therapeutic perspective," *Clinical Science*, vol. 122, no. 5, pp. 203–214, 2012.

[28] L. R. Watkins and S. F. Maier, "Glia: a novel drug discovery target for clinical pain," *Nature Reviews Drug Discovery*, vol. 2, no. 12, pp. 973–985, 2003.

[29] Y. Wang, A. M. Schmeichel, H. Iida, J. D. Schmelzer, and P. A. Low, "Enhanced inflammatory response via activation of NF-$\kappa$B in acute experimental diabetic neuropathy subjected to ischemia-reperfusion injury," *Journal of the Neurological Sciences*, vol. 247, no. 1, pp. 47–52, 2006.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2013, Article ID 164817, 5 pages
http://dx.doi.org/10.1155/2013/164817

*Research Article*

# Anti-Inflammatory Activity of Water-Soluble Polysaccharide of *Agaricus blazei* Murill on Ovariectomized Osteopenic Rats

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Received 5 October 2013; Accepted 28 October 2013

Academic Editor: Chunchao Han



In the present study, we investigated the anti-inflammatory activity of water-soluble polysaccharide of *Agaricus blazei* Murill (WSP-AbM) on ovariectomized osteopenic rats. The rats were administered orally WSP-AbM (200 mg/kg BW) for 8 weeks. Subsequent serum maleic dialdehyde (MDA) level, total antioxidant status (TAOS), nuclear factor kappa B (NF-$\kappa$B) level, polymorphonuclear (PMN) cells level, interleukin-1$\beta$ (IL-1$\beta$) level, inducible nitric oxide synthase (iNOS) level, tumor necrosis factor-$\alpha$ (TNF-$\alpha$) level, adhesion molecule (ICAM-1), and cyclooxygenase-2 (COX-2) were determined by enzyme linked immunosorbent assay (ELISA) and immunohistochemistry, respectively. WSP-AbM administration markedly ($P < 0.05$) decreased serum IL-1$\beta$ and TNF-$\alpha$ levels and the expressions of ICAM-1, COX-2, and iNOS NF-$\kappa$B compared with OVX rats. WSP-AbM administration alsomarkedly ($P < 0.05$) decreased PMN infiltration. In conclusion, we observed that WSP-AbM supplementation had anti-inflammatory effects in a model of osteoporosis disease.

## 1. Introduction

Several inflammatory diseases have been associated to bone resorption. Chronic inflammatory diseases are associated with a significant risk for secondary osteoporosis and fractures [1, 2]. Current evidence suggests that the osteoporosis developed during chronic inflammation may result from the inhibition of bone formation and is associated with systemic overproduction of proinflammatory mediators, such as cytokines [3, 4]. Therefore, searching for effective drugs which can control the inflammation of osteopenia is of great significance for patients with osteopenia.

The basidiomycete *Agaricus blazei* Murill (AbM), popularly known as "sun mushroom," is native to Brazil and widely grown in Japan and China because of its medicinal properties. It is widely used for nonprescript, medicinal purposes, both as an edible mushroom and in the form of extracts [5]. AbM has traditionally been used for the prevention of a range of diseases, including cancer, hepatitis, atherosclerosis, hypercholesterolemia, diabetes, and dermatitis [6]. Considering all the effects found for the immune stimulating activity of WSP-AbM and its relation to many physiological processes, the aim of this study was to evaluate the anti-inflammatory effect of WSP-AbM in ovariectomized osteopenic rats.

## 2. Material and Methods

*2.1. Preparation of the Water-Soluble Polysaccharide of Agaricus blazei* Murill *(WSP-AbM).* The fermented mushroom of AbS was produced by *Coprinus comatus* [7]. The aqueous extraction was performed by adding 100 mL boiling water to 10 g air-dried mycelium. The infusion stood at room temperature for 30 minutes. After cooling and filtration, the extract was concentrated to one-tenth of the volume and precipitated with 4 vol of 95% ethanol at 4°C for 24 h. The precipitate collected by centrifugation was deproteinated by proteinase digestion, followed by exhaustive dialysis with water for 48 h. Then the concentrated dialyzate was precipitated with 4 vol of 95% EtOH at 4°C for 24 h. The precipitate was washed with

absolute ethanol, acetone, and ether, respectively, giving the water-soluble polysaccharide of *Agaricus blazei* Murill (WSP-AbM).

*2.2. Experimental Design.* Thirty female Wistar rats (2 months old and weighing 225 ± 25 g) were used in the study. Good laboratorial animal practice was performed according to the International Standards for Animal Experimentation. The rats were randomly divided into three groups of animals, two ovariectomized (OVX) and another group which was given a sham operation (control) [8]. Then groups 1 ($n$ = 10, sham) and 2 ($n$ = 10, OVX) were administered orally vehicle (PBS), and group 3 ($n$ = 10) was administered orally WSP-AbM (WSP-AbM at 200 mg/kg/day) for 8 weeks. At sacrifice, the serum was obtained by centrifugation using a serum separator tube and then stored immediately at −20°C to estimate inflammatory cells and inflammatory mediators.

*2.3. Estimation of Maleic Dialdehyde (MDA).* MDA was determined with thiobarbituric acid (TBA) using the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute). Total protein content of the samples was analyzed using coomassie blue assay (Nanjing Jiancheng Bioengineering Institute).

*2.4. Estimation of Serum IL-1β and TNF-α Level.* Serum samples were disintegrated in 5 volumes of ice-cold RIPA buffer. After incubation on ice for 30 minutes, samples were centrifuged twice at 20,000 Xg for 15 minutes at 4°C. The resulting supernatants were used for assay. The concentration of IL-1$\beta$ and TNF-$\alpha$ was determined using a commercial ELISA kit (Shanghai Jinma Biological Technology, Inc., China) following the manufacture's instruction.

*2.5. Estimation of Serum COX-2, iNOS, and ICAM-1 Levels.* The procedures were processed according to the protocols recommended for COX-2, iNOS, and ICAM-1 immunohistochemistry kits (Hengdabaisheng Biotechnology, Beijing, China).

*2.6. Quantification of NF-κB Activity.* Activated NF-κB was quantified via ELISA technique using the PathScan Phospho-NF-κB p65 (Ser536) Sandwich ELISA Antibody Pair (Shanghai Yubo Biological Technology, Inc., China), following the manufacture's instruction.

*2.7. Measurement of Total Antioxidant Status.* The total antioxidant status (TAOS) of serum was determined as previously described by Laight et al. [9]. The increase of absorbance at 405 nm was measured by a microplate reader (Shanghai Xunda Medical Technology, Inc., China).

*2.8. Statistical Analysis.* All data were analyzed by a one-way analysis of variance, and the differences between means were established by Duncan's multiple-range test. The data are shown as the means ± SEM. The significant level of 5% ($P < 0.05$) was used as the minimum acceptable probability for the difference between the means.

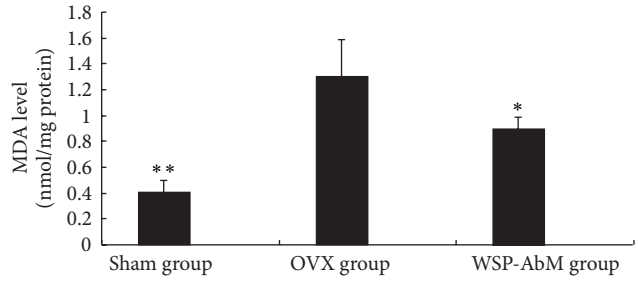


Figure 1: Effect of WSP-AbM on MDA level. Values are shown as means ± SEM. $^{*}P < 0.05$ versus OVX group and $^{**}P < 0.01$ versus OVX group.

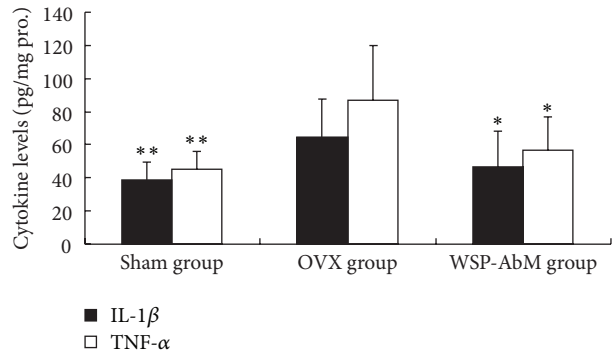


Figure 2: Effect of WSP-AbM on hepatic TNF-$\alpha$ and IL-1$\beta$ levels. Values are shown as means ± SEM. $^{*}P < 0.05$ versus OVX group and $^{**}P < 0.01$ versus OVX group.

## 3. Results

*3.1. The Effect of WSP-AbM on MDA.* In order to evaluate the effect of WSP-AbM on serum lipid peroxidation, we determined the MDA levels. The serum from sham-operated controls contained low MDA level. MDA levels in ovariectomized group were significantly higher than those in control group ($P < 0.01$). Rats treated with WSP-AbM significantly ($P < 0.05$) decreased ethanol-induced MDA elevation in serum (Figure 1).

*3.2. The Effect of WSP-AbM on TNF-α and IL-1β.* The overexpression of TNF-$\alpha$ and IL-1$\beta$ induced by OVX was evaluated at protein levels by ELISA. Figure 2 shows that OVX significantly increased protein concentration of IL-1$\beta$ in the serum. WSP-AbM treatment decreased the level of IL-1$\beta$ by 27.3% as compared to the OVX group ($P < 0.05$). As shown in Figure 2, the levels of TNF-$\alpha$ elevated significantly after OVX-induced osteoporosis. WSP-AbM suppressed this response ($P < 0.05$).

*3.3. The Effect of WSP-AbM on COX-2, iNOS, and ICAM-1 Levels.* Rats subjected to OVX-induced osteoporosis showed typical markers of inflammation upregulation of adhesion molecule and induction of prooxidative enzymes (COX-2 and iNOS). The protein expressions of COX-2 in OVX group significantly increased compared with those of the

Table 1: Effect of WSP-AbM on ICAM-1, iNOS, and COX-2 protein production (number of immunopositive/mm$^2$).

| Different groups | COX-2 | iNOS | ICAM-1 |
|---|---|---|---|
| Sham group | 11.00 ± 6.06** | 11.40 ± 4.22** | 21.23 ± 7.23** |
| OVX group | 70.11 ± 8.22 | 70.28 ± 9.22 | 121.12 ± 35.35 |
| WSP-AbM group | 42.11 ± 4.55** | 35.20 ± 5.00* | 99.40 ± 21.20* |

Values are shown as means ± SEM. $^*P < 0.05$ versus OVX group and $^{**}P < 0.01$ versus OVX group.

Table 2: Effect of WSP-AbM on TAOS activity ($\mu$M L-ascorbate).

| Different groups | TAOS activity ($\mu$M L-ascorbate) |
|---|---|
| Sham group | 28.41 ± 3.17** |
| OVX group | 80.33 ± 9.32 |
| WSP-AbM group | 56.35 ± 4.33** |

Values are shown as means ± SEM. $^{**}P < 0.01$ versus OVX group.

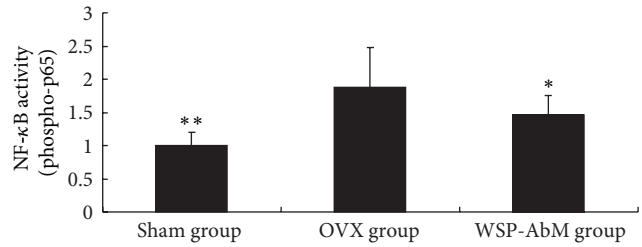


Figure 3: Effect of WSP-AbM on NF-$\kappa$B activity. Values are shown as means ± SEM. $^*P < 0.05$ versus OVX group and $^{**}P < 0.01$ versus OVX group (control group is set to 1).

sham group. The protein expressions of COX-2 decreased in WSP-AbM-treated groups (Table 1). In this study, WSP-AbM suppressed OVX-induced iNOS expression.

The protein expressions of ICAM-1 in the OVX group significantly increased compared with those of the sham group. WSP-AbM treatment decreased the level of ICAM-1 as compared to the OVX group ($P < 0.05$) (Table 1).

We hypothesized that WSP-AbM could potentially produce the above beneficial effects through the decreased expression of NF-$\kappa$B. As shown in Figure 3, OVX significantly induced the activated NF-$\kappa$B above control levels, and as hypothesized, WSP-AbM significantly suppressed this response. It is consistent with the results presented in Figure 2.

*3.4. Effects of WSP-AbM on Total Antioxidant Status (TAOS).* The results of serum TAOS are shown in Table 2. TAOS in the OVX-treated group were significantly ($P < 0.01$) higher than those in the sham group. Those in the WSP-AbM-treated groups were significantly lower than those in the OVX-treated group ($P < 0.01$).

## 4. Discussion

Osteoporosis is a generalized metabolic disease characterized by progressive loss of elements of bone tissue, leading to bone fragility and increasing the risk of fracture [10, 11]. Current drugs used for the treatment of osteoporosis may exert adverse side effects as jaw osteonecrosis or upper gastrointestinal diseases for bisphosphonates [12]. Therefore, naturally occurring bioactive dietary compounds endowed with positive effects on bone health represent an attractive alternative for managing osteoporosis. In the current experiments, we used WSP-AbM in an in vivo animal model of OVX osteopenic rats. Our results showed that supplementation of WSP-AbM attenuated inflammatory response in OVX rats.

MDA is a major reactive aldehyde that appears during the final stages of lipid peroxidation of biological membrane polyunsaturated fatty acid [13]. MDA activity is commonly used as an indicator of tissue damage involving a series of chain reactions [14]. Accordingly, we sought to determine whether WSP-AbM would provide antioxidation by measuring the MDA level. The serum from sham-control animals contained low MDA level. MDA levels in OVX group were significantly higher than those in sham group l ($P < 0.01$). MDA level in WSP-AbM group was significantly lower than that in OVX group ($P < 0.05$) (Figure 1). Induction of oxidative stress was identified as key element in the pathophysiology of osteoporosis [15]. These results indicated that the free radicals being released in the serum were effectively scavenged by WSP-AbM, which may also account for its anti-inflammatory properties.

Activation of the inflammatory cascade was identified as another key element in the pathophysiology of osteoporosis [15]. Abnormal metabolism of cytokine is a major feature of osteoporosis. The expressions of TNF-$\alpha$ and IL-1$\beta$ were found to be enhanced in both animal model and patients with osteoporosis [16]. In this study, WSP-AbM treatment significantly attenuated OVX-induced TNF-$\alpha$ and IL-1$\beta$ expression at protein level. Thus, we hypothesized that the protective effect of WSP-AbM on OVX-induced osteoporosis was at least in part mediated by its anti-inflammation.

NO produced by inducible NOS (iNOS) plays crucial role in the development of inflammatory osteoporosis [17, 18]. And the iNOS is induced by cytokines such as IFN-c and TNF-$\alpha$ [19–21]. Therefore, we wondered whether WSP-AbM, which inhibits cytokines expression, had any positive therapeutic effects on iNOS. The results showed that WSP-AbM treatment inhibited the development of inflammation and suppressed cytokines-induced iNOS expression.

NO also activates COX enzymes leading to a marked increase in PGE2 production [22]. COX-2 is primarily responsible for the increased PGE2 production during inflammation, and PGE2 is generally considered to be a proinflammatory agent [23, 24]. As shown in Table 2,

WSP-AbM treatment significantly decreased the expression of COX-2 protein in OVX rats.

The active mode of WSP-AbM in the prevention of OVX-induced osteoporosis also involves inhibiting ICAM-1 expression. Cell adhesion molecule ICAM-1 is inducible by both NF-$\kappa$B activation and inflammatory cytokines such as TNF-$\alpha$ and IL-1$\beta$ [25, 26]. Expression of ICAM-1 on hepatocytes correlates with the degree of osteoporosis [27]. In the OVX-induced osteoporosis model, we detected overexpression of ICAM-1, which was decreased in the WSP-AbM-treated group. This indicated that WSP-AbM could suppress lymphocyte adhesion to the endothelium and inhibition of the migration of lymphocytes from blood vessels and penetration of the subendothelium.

Many reports showed that OVX-induced reactive oxygen species can activate redox-sensitive nuclear factor NF-$\kappa$B [28]. NF-$\kappa$B activation triggers the induction of inflammatory genes and plays an important role in initiation and progression of OVX disease [29]. As hypothesized, WSP-AbM significantly suppressed this response. It was in line with the previous study [28, 29].

The total antioxidant status (TAOS) is an indication of $O_2^-$ and other oxidant species. We measured TAOS activity as an indirect indication of the formation of $O_2^-$ and other oxidant species. $O_2^-$ is produced by polymorphonuclear leukocytes and macrophages from the enzyme activity of NADPH oxidase and xanthine oxidase at inflammatory sites. The WSP-AbM groups had the lowest level of TAOS activity in comparison to the OVX group. We hypothesized that WSP-AbM produces anti-inflammatory effect through decreasing the levels of TAOS activities.

In conclusion, the present data indicate that WSP-AbM supplementation could restrain the inflammation caused by OVX. Activation of NF-$\kappa$B plays an important role in the pathogenesis of osteoporosis by the upregulation of TNF-$\alpha$, IL-1$\beta$, ICAM-1, iNOS, and COX-2 expressions. WSP-AbM reduced TNF-$\alpha$, IL-1$\beta$, ICAM-1, iNOS, and COX-2 expressions and osteoporosis by the inhibition of NF-$\kappa$B activation. Hence, the present results suggests for the first time, the anti-inflammatory effects of WSP-AbM in a model of osteoporosis disease.

## Conflict of Interests

The authors declare that there is no conflict of interests.

## Acknowledgments

This study was supported by the Foundation of Department of Education of Heilongjiang Province (no. 14521635). Peng Wang, Xiao-Tao Li, and Lei Sun are co-first authors.

## References

[1] B. A. Michel, D. A. Bloch, and J. F. Fries, "Predictors of fractures in early rheumatoid arthritis," *Journal of Rheumatology*, vol. 18, no. 6, pp. 804–808, 1991.

[2] B. A. Michel, D. A. Bloch, F. Wolfe, and J. F. Fries, "Fractures in rheumatoid arthritis: an evaluation of associated risk factors," *Journal of Rheumatology*, vol. 20, no. 10, pp. 1666–1669, 1993.

[3] H. Sakurai, H. Kohsaka, M.-F. Liu et al., "Nitric oxide production and inducible nitric oxide synthase expression in inflammatory arthritides," *Journal of Clinical Investigation*, vol. 96, no. 5, pp. 2357–2363, 1995.

[4] G. Dijkstra, H. Moshage, H. M. van Dullemen et al., "Expression of nitric oxide synthases and formation of nitrotyrosine and reactive oxygen species in inflammatory bowel disease," *The Journal of Pathology*, vol. 186, pp. 416–421, 1998.

[5] S. P. Wasser and A. L. Weis, "Therapeutic effects of substances occurring in higher basidiomycetes mushrooms: a modern perspective," *Critical Reviews in Immunology*, vol. 19, no. 1, pp. 65–96, 1999.

[6] C.-H. Hsu, Y.-L. Liao, S.-C. Lin, K.-C. Hwang, and P. Chou, "The mushroom Agaricus blazei Murill in combination with metformin and gliclazide improves insulin resistance in type 2 diabetes: a randomized, double-blinded, and placebo-controlled clinical trial," *Journal of Alternative and Complementary Medicine*, vol. 13, no. 1, pp. 97–102, 2007.

[7] C. Han, J. Yuan, Y. Wang, and L. Li, "Hypoglycemic activity of fermented mushroom of Coprinus comatus rich in vanadium," *Journal of Trace Elements in Medicine and Biology*, vol. 20, no. 3, pp. 191–196, 2006.

[8] Y. Jiang, J. J. Zhao, B. H. Mitlak, O. Wang, H. K. Genant, and E. F. Eriksen, "Recombinant human parathyroid hormone (1–34) [teriparatide] improves both cortical and cancellous bone structure," *Journal of Bone and Mineral Research*, vol. 18, no. 11, pp. 1932–1941, 2003.

[9] D. W. Laight, P. T. Gunnarsson, A. V. Kaw, E. E. Änggård, and M. J. Carrier, "Physiological microassay of plasma total antioxidant status in a model of endothelial dysfunction in the rat following experimental oxidant stress in vivo," *Environmental Toxicology and Pharmacology*, vol. 7, no. 1, pp. 27–31, 1999.

[10] W. A. Peck, "Consensus development conference: diagnosis, prophylaxis, and treatment of osteoporosis," *American Journal of Medicine*, vol. 94, no. 6, pp. 646–650, 1993.

[11] O. Johnell and J. Kanis, "Epidemiology of osteoporotic fractures," *Osteoporosis International*, vol. 16, no. 2, pp. S3–S7, 2005.

[12] R. Rizzoli and J.-Y. Reginster, "Adverse drug reactions to osteoporosis treatments," *Expert Review of Clinical Pharmacology*, vol. 4, no. 5, pp. 593–604, 2011.

[13] C. E. Vaca, J. Wilhelm, and M. Harms-Ringdahl, "Interaction of lipid peroxidation products with DNA. A review," *Mutation Research*, vol. 195, no. 2, pp. 137–149, 1988.

[14] H. Ohkawa, N. Ohishi, and K. Yagi, "Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction," *Analytical Biochemistry*, vol. 95, no. 2, pp. 351–358, 1979.

[15] R. Will, R. Palmer, A. K. Bhalla, F. Ring, and A. Calin, "Osteoporosis in early ankylosing spondylitis: a primary pathological event?" *The Lancet*, vol. 2, no. 8678-8679, pp. 1483–1485, 1989.

[16] J. Pfeilschifter, C. Wuster, M. Vogel, B. Enderes, R. Ziegler, and H. W. Minne, "Inflammation-mediated osteopenia (IMO) during acute inflammation in rats is due to a transient inhibition of bone formation," *Calcified Tissue International*, vol. 41, no. 6, pp. 321–325, 1987.

[17] K. E. Armour, R. J. van't Hof, P. S. Grabowski, D. M. Reid, and S. H. Ralston, "Evidence for a pathogenic role of nitric oxide in inflammation-induced osteoporosis," *Journal of Bone and Mineral Research*, vol. 14, no. 12, pp. 2137–2142, 1999.

[18] K. J. Armour, K. E. Armour, R. J. van't Hof et al., "Activation of the inducible nitric oxide synthase pathway contributes to

inflammation-induced osteoporosis by suppressing bone formation and causing osteoblast apoptosis," *Arthritis & Rheumatism*, vol. 44, pp. 2790–2796, 2001.

[19] G. Sass, S. Heinlein, A. Agli, R. Bang, J. Schümann, and G. Tiegs, "Cytokine expression in three mouse models of experimental hepatitis," *Cytokine*, vol. 19, no. 3, pp. 115–120, 2002.

[20] B. Jaruga, F. Hong, W.-H. Kim, and B. Gao, "IFN-$\gamma$/STAT1 acts as a proinflammatory signal in T cell-mediated hepatitis via induction of multiple chemokines and adhesion molecules: a critical role of IRF-1," *American Journal of Physiology—Gastrointestinal and Liver Physiology*, vol. 287, no. 5, pp. G1044–G1052, 2004.

[21] S. Fujikura, H. Mizuhara, Y. Miyazawa, H. Fujiwara, and K. Kaneda, "Kinetics and localization of lymphoblasts that proliferate in the murine liver after concanavalin a administration," *Biomedical Research*, vol. 17, no. 2, pp. 129–139, 1996.

[22] M. Mandai, N. Yoshimura, M. Yoshida, M. Iwaki, and Y. Honda, "The role of nitric oxide synthase in endotoxin-induced uveitis: effects of $N^G$-nitro L-arginine," *Investigative Ophthalmology and Visual Science*, vol. 35, no. 10, pp. 3673–3680, 1994.

[23] S. Endres, H.-J. Fülle, B. Sinha et al., "Cyclic nucleotides differentially regulate the synthesis of tumour necrosis factor-$\alpha$ and interleukin-1$\beta$ by human mononuclear cells," *Immunology*, vol. 72, no. 1, pp. 56–60, 1991.

[24] J.-Y. Guo, C.-Y. Li, Y.-P. Ruan et al., "Chronic treatment with celecoxib reverses chronic unpredictable stress-induced depressive-like behavior via reducing cyclooxygenase-2 expression in rat brain," *European Journal of Pharmacology*, vol. 612, no. 1–3, pp. 54–60, 2009.

[25] B. Zingarelli, M. Sheehan, and H. R. Wong, "Nuclear factor-$\kappa$B as a therapeutic target in critical care medicine," *Critical Care Medicine*, vol. 31, no. 1, pp. S105–S111, 2003.

[26] S. J. Wertheimer, C. L. Myers, R. W. Wallace, and T. P. Parks, "Intercellular adhesion molecule-1 gene expression in human endothelial cells. Differential regulation by tumor necrosis factor-$\alpha$ and phorbol myristate acetate," *Journal of Biological Chemistry*, vol. 267, no. 17, pp. 12030–12035, 1992.

[27] B. Krempien, S. Vukicevic, M. Vogel, A. Stavljenic, and R. Buchele, "Cellular basis of inflammation-induced osteopenia in growing rats," *Journal of Bone and Mineral Research*, vol. 3, no. 5, pp. 573–582, 1988.

[28] V. Iotsova, J. Caamaño, J. Loy, Y. Yang, A. Lewin, and R. Bravo, "Osteopetrosis in mice lacking NF-$\kappa$B1 and NF-$\kappa$B2," *Nature Medicine*, vol. 3, no. 11, pp. 1285–1289, 1997.

[29] D. V. Novack, "Role of NF-$\kappa$B in the skeleton," *Cell Research*, vol. 21, no. 1, pp. 169–182, 2011.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2013, Article ID 842619, 6 pages
http://dx.doi.org/10.1155/2013/842619

*Review Article*

# The Medicinal Values of Culinary-Medicinal Royal Sun Mushroom (*Agaricus blazei Murrill*)

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Received 5 September 2013; Accepted 18 September 2013

Academic Editor: Jianyou Guo



*Agaricus blazei* Murrill (ABM), a mushroom native to Brazil, is a basidiomycete brown fungus, which is popularly known as "*Cogumelo do Sol*" in Brazil or "*Himematsutake*" in Japan, and there has been a prominent increase in the use of ABM for therapeutic and medicinal purposes. ABM is useful against a variety of diseases like cancer, tumor, chronic hepatitis, diabetes, atherosclerosis, hypercholesterolemia, and so on. In this review, we demonstrated various pharmacological effects of ABM, so that we can use different effects of ABM against different diseases and provide reference for the study of ABM in the future.

## 1. Introduction

Mushrooms have been used in humans' food since ancient times, which are low in calories and high in minerals, vitamins, fibers, and essential amino acids. In recent years, there has been a significant increase in the consumption of mushroom due to an increasing number of studies identifying the therapeutic properties of the substances isolated from various species of these fungi [1].

*Agaricus blazei* Murrill (or *A. brasiliensis*), a mushroom of Brazilian origin, is widely used for nonprescript, medicinal purposes, both as an edible mushroom and in the form of extracts, which has been used as a health care product for the prevention of a wide range of illnesses including cancer, tumor, chronic hepatitis, diabetes, atherosclerosis, and hypercholesterolemia (Table 1). With the development of scientific researches, more and more scientific research have studied the chemical constituents and pharmacological effects of *A. brasiliensis*. The major chemical compounds include polysaccharide, protein, lectins, amino acid, vitamin, and sterols.

After the *in vivo* and *in vitro* studies, the pharmacological effects of *A. brasiliensis* including antitumor, antiviral, anti-inflammatory, liver protection, antidiabetic, antihyperlipidemic, antiatherosclerosis, antiallergic, and immunomodulating were found. Although the clinical research about pharmacological effects of *A. brasiliensis* is less, *A. brasiliensis* as a complementary and alternative medicine is widely used.

## 2. Medicinal Values of *A. brasiliensis*

*2.1. Anticancer Activity. Agaricus brasiliensis*, a medicinal edible fungus, is widely studied and used because of its significant anticancer activity. In Japan, researchers demonstrated anticancer and immunostimulant effects of *A. brasiliensis* extracts experimentally, and due to the improving consumption of this mushroom in recent years, a considerable effort investigated the putative effects with interesting but still insufficient clinical studies. Some reports showed that polysaccharide is the main component of *A. brasiliensis* for antitumor [2–4].

*2.1.1. Studies in Animals.* Polysaccharide antitumoral activity has been evaluated most often against allogenic sarcoma 180 in CD-I mice [5]. *A. brasiliensis* (Himematsutake) has stronger antitumor activity against Sarcoma 180 in mice than do polysaccharides from *Ganoderma lucidum*, *Lentinus edodes*, and *Coriolus versicolor* [6]. Mizuno et al. [6] studied the against antitumor polysaccharide Sarcoma 180 from the mycelium of liquid-cultured *A. brasiliensis*. But the isolated polysaccharide did not react with antibodies of antitumor polysaccharides such as lentinan, gliforan, and FIII-2-b, which is one of the antitumor polysaccharides from *A. brasiliensis*. Moreover, the analyses of $^{13}$C-NMR and GC-MS suggested that this polysaccharide was preliminarily glucomannan with a main chain of $\beta$-l,2-1inked

Table 1: Medicinal values and active compounds of *A.brasiliensis*.

| Medicinal value | Active compounds | References |
|---|---|---|
| Anticancer activity | Polysaccharides agaritine | [2–10] |
| Antiviral activity | Polysaccharides<br>Protein polysaccharide | [11–13] |
| Liver protection | Aqueous extract | [14–20] |
| Immunomodulating effect | Polysaccharides | [21–24] |
| Antidiabetic effect | $\beta$-glucans and their enzymatically hydrolyzed oligosaccharides (AO) | [25–28] |
| Antileishmaniasis effect | Aqueous extract | [29–31] |

D-mannopyranosyl residues and $\beta$-D-glucopyranosyl-3-O-13-D-glucopyranosyl residues as a side chain [7].

However, some reports indicated that agaritine and its derivatives exerted antitumor activity against leukemic cells, mainly U937 cells [8, 9]. Agaritine was fractionated by HPLC from a hot water extract of ABM powder, and the structure was determined by NMR and MS analyses. This compound inhibited the proliferation of leukemic cell lines, especially suppressing the viability of U937 cells *in vitro* [8]. Therefore, the antitumor substances of ABM remain to be further researched.

*2.1.2. Clinical Studies.* It has been reported that 100,000–300,000 kg of the dried body of *A. blazei* is produced every year in Japan, and about 300,000–500,000 persons assume the 3–5 g three times a day by a typical hot water extract of *A. blazei* as an adjuvant with cancer chemotherapy drugs for the prevention or treatment of cancer.

*2.2. Antiviral Activity.* The pharmacological effects of *Agaricus brasiliensis* have been mainly related to the presence of polysaccharides and protein polysaccharide complexes [11]. Faccin et al. [12] reported that the extracts of fruiting bodies of ABM, including aqueous and alcohol extracts, and an isolated against polysaccharide from this species displayed antiviral activity poliovirus type 1. de Sousa Cardozo et al. [13] reported the chemical modification of a polysaccharide extracted from *A. brasiliensis* mycelia to obtain its sulfated derivative (MI-S), which presented a promising inhibitory activity against HSV-1 (KOS and 29R (acyclovir-resistant) strains) and HSV-2 strain 333. Furthermore, the sulfated polysaccharide also presented synergistic antiviral effect with acyclovir.

*2.3. Liver Protection.* Hepatic fibrosis is caused by chronic damage to the liver in union with the progressive accumulation of fibrillar extracellular matrix proteins [32–34]. The main causes of hepatic fibrosis in humans include infection by hepatitis B and C, alcohol abuse, and nonalcohol steatohepatitis, and liver cirrhosis can be induced by carbon tetrachloride ($CCL_4$) [35, 36]. A few studies have researched that *A. brasiliensis* extract could ameliorate or abrogate $CCL_4$-induced liver injury in rats [14, 15].

*2.3.1. Studies in Animals.* Chen et al. [16] have demonstrated that *A. brasiliensis* extract might serve as an adjuvant in improving the efficacy of hepatitis B vaccines *in vivo.* The results showed that not only a significant increase in the HBcAg-specific antibody response was observed, but also T cell proliferation was observed in mice which received HBcAg DNA vaccine plus *A. brasiliensis* extract [16].

*2.3.2. Clinical Studies.* Hsu et al. [17] performed a 1-year open-label pilot study to observe whether *A. brasiliensis* extract improves liver function in patients with hepatitis B. They gave the four enrolled patients *A. brasiliensis* extract of 1500 mg daily for 12 months and measured the level of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). At the end of the study, the mean level of AST and ALT decreased from 246.0 (± standard deviation (SD) 138.9) to 61.3 (± SD 32.6) IU/L and 151.0 (± SD 86.9) to 46.1 (± SD 22.5) IU/L, respectively [17]. Although the result of this study showed that *A. brasiliensis* extract can normalize liver function of the 4 patients, this study is just a small sample research.

In addition, Grinde et al. [18] reported the effect on gene expression in peripheral blood cells from four chronic hepatitis C patients, using global (29 k) oligo-based, single channel microarrays. After dates being analyzed, the results suggested that the $\beta$-glucan part of the *A. brasiliensis* extract was not transported into the blood in appreciable quantities. And although the average ($n = 5$) titre of virus was slightly lower after one week on *A. brasiliensis*, the difference was clearly not significant [18]. So, this result of the study showed that $\beta$-glucan of the *A. brasiliensis* extract cannot treat HCV. However, one study evaluated the clinical effects and safety on 20 volunteers (50% of men) with elevated $\gamma$-GTP activity of *A. blazei* condensed liquid (*Agaricus* mushroom extract, ABCL) in the treatment of C-hepatitis. Decreasing effect for serum $\gamma$-GTP activity was found in 80% of the patients in both sexes after these patients received the ABCL orally, twice a day, for 8 weeks, without any toxicological findings and other side effects [19]. Additionally, Mukai et al. [20] reported three cases of patients with advanced cancer who showed severe hepatic damage, and two of whom died of fulminant hepatitis after taking *A. brasiliensis* extract. Reporters demonstrated that a strong causal relationship between the *A. brasiliensis* extract and liver damage was suggested and, at least, taking the *A. brasiliensis* extract made the clinical decision-making process much more complicated, although several other factors cannot be completely ruled out as the causes of liver damage [20].

*2.4. Immunomodulating Effect.* Polysaccharide is an immunologic adjuvant, which not only can activate the activity of T cells, B cells, NK cells, and other immune cells but also can promote the synthesis of IL-1, IL-2, TNF-$\alpha$, IFN-$\gamma$, and NO, regulating the formation of body's antibodies and complement. *A. brasiliensis* contains compounds such as $(1 \rightarrow 3)$, $(1 \rightarrow 6)$-$\beta$-glucans (Figure 1), $(1 \rightarrow 3)$-$\alpha$-glucans, and protein-polysaccharide complexes, which can enhance *in vivo* and *in vitro* cell-mediated immune responses and act as biological response modifiers [21, 22].

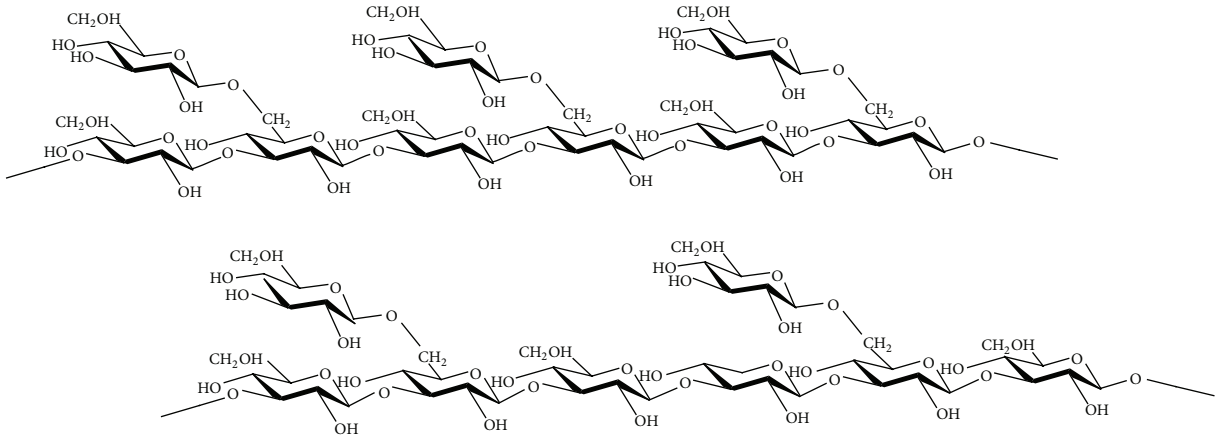

Figure 1: $\beta$-(1,3-1,6)-D-Glucan.

*2.4.1. Studies in Animals.* Lin et al. [23] have established leukemia mice through the injection of WEHI-3 cells and chronically treated mice with *A. brasiliensis*. In their study, results showed that *A. brasiliensis* can promote immune responses in leukemia mice *in vivo* and also can promote T cell proliferation. Furthermore, the *A. brasiliensis* extract significantly enhanced both NK cell activities and phagocytosis of macrophages [23].

*2.4.2. Clinical Studies.* However, there was a randomized clinical trial on elderly women to ascertain the effects of AbM intake on serum levels of IL-6, IFN-$\gamma$, and TNF-$\alpha$ in community-living seniors [24]. After the study period, no changes from baseline were detectable for any parameter in either group, receiving placebo or AbM dry extract with 900 mg/day for 60 days. Therefore, it showed that AbM extract had no modulating effect on IL-6, IFN-c, or TNF-a levels in elderly females [24].

*2.5. Antidiabetic Effect.* *Agaricus brasiliensis* is rich in polysaccharides and protein, especially $\beta$-glucans. Kim et al. [25] demonstrated that $\beta$-glucans and their enzymatically hydrolyzed oligosaccharides (AO) from *A. brasiliensis* show the activities of antihyperglycemic, antihypertriglyceridemic, antihypercholesterolemic, and antiarteriosclerotic indicating antidiabetic activity as a whole in diabetic rats. In this study, diabetic rats were divided into four groups, including normal control, diabetic control, treated group I ($\beta$-glucans), and treated group II (AO), to contrast the different changes of their body weights. The data suggested that both $\beta$-glucans and AO might promote insulin secretion from islets as well as the viability and proliferation of islets in diabetic or normal rats.

In addition, several studies demonstrated that *A. brasiliensis* had an effect on streptozotocin-induced diabetic rats [26–28]. Oxidative stress induced by hyperglycemia possibly causes the dysfunction of pancreatic $\beta$-cells and various forms of tissue damage in patients with diabetes mellitus. Niwa et al. [28] researched the antidiabetic efficacy and hypoglycemic mechanisms of *Ipomoea batatas* and *A. brasiliensis* in streptozotocin-induced diabetic rats. The results of the study suggested hypoglycemic effects of *Ipomoea batatas* or *A. brasiliensis* due to their suppression of oxidative stress and proinflammatory cytokine production followed by improvement of pancreatic $\beta$-cells mass [28].

*2.6. Antileishmanial Effect.* Leishmaniasis is a flock of vector-transmitted diseases that are endemic in many tropical and subtropical countries. The current treatment for leishmaniasis has certain side effects, and some drugs are of high cost for the majority of patients. In recent years, *A. brasiliensis* was demonstrated to have antileishmanial activity, and thereinto an *in vitro* antileishmanial activity against *L. amazonens is*, *L. chagasi*, and *L. major* was demonstrated for an *A. blazei* water extract [29]. Valadares et al. [30] studied the therapeutic efficacy induced by the oral administration of *A. brasiliensis* against *Leishmania amazonensis*. The results showed that mice treated with the *A. brasiliensis* presented a 60% reduction in the inflammation of infected footpads as compared to untreated control-infected mice. These treated animals produced significantly higher levels of interferon gamma (IFN-$\gamma$) and nitric oxide (NO), higher levels of parasite-specific IgG2a isotype antibodies, and lower levels of IL-4 and IL-10 in the spleen and lymph node cell cultures than did the controls [30]. In addition, Valadares et al. [31] used five fractions obtained from *A. brasiliensis* water extract to treat BALB/c mice infected with *Leishmania chagasi in vivo* (Figure 2). The results suggested that the use of Fab5 (molecules >100,000 Da) or *A. brasiliensis*, as compared to control groups, resulted in significant reduced parasite burdens in the liver, spleen, and draining lymph nodes of the infected animals.

## 3. Other Effects

There are not only these pharmacological effects of *A. brasiliensis*, which are described previously, but also other beneficial effects. The chloroform-soluble extract of *A. brasiliensis* inhibited IL-6 production in PMA plus A23187-induced BMMCs (bone marrow-derived mast cells) to express the

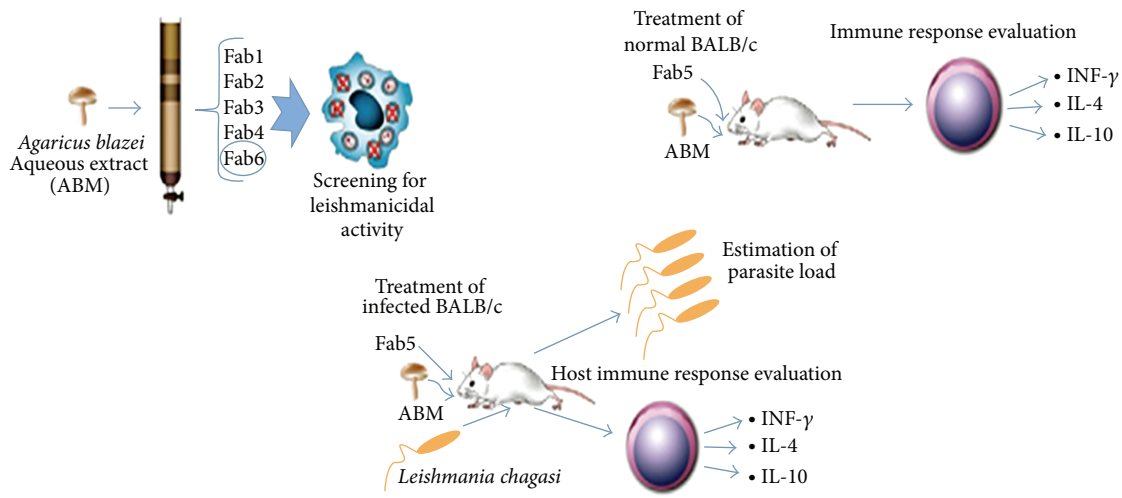


Figure 2: Reference schematic diagram of Valadares et al. using the five fractions obtained from ABM water extract to treat BALB/c mice infected with *Leishmania chagasi*.

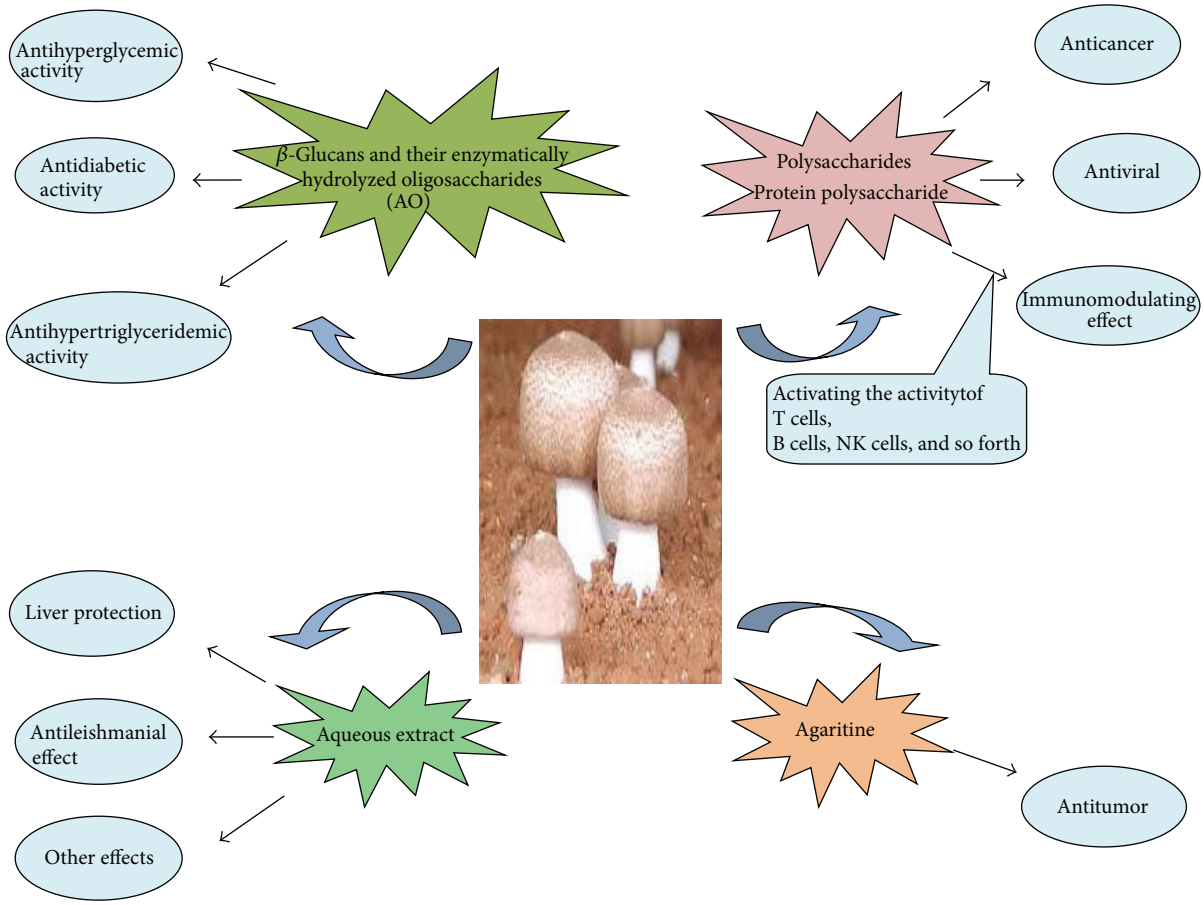


Figure 3: Medicinal values and active compounds of *A. brasiliensis*.

anti-inflammatory and antiallergic effects [37]. One study, the first *in vivo* study, showed that *A. brasiliensis* can enhance local and systemic inflammation, upregulate proinflammatory molecules, and enhance leukocyte homing to atherosclerosis sites without affecting the lipoprotein profile [38]. The polysaccharides of *A. brasiliensis* have antitumor, antiviral, and immunomodulating effects as described above. Besides, one study indicated that *A. brasiliensis* polysaccharides could be useful in promoting burn wound healing [39].

There is a clinical study stating that Administration of γ-aminobutyric acid (GABA) enriched *A. blazei* (AG-GABA) to mild hypertensive human subjects showed that

both systolic and diastolic blood pressure values decreased to statistically significant levels [40]. Maybe there are also many other effects of *A. brasiliensis*, which are not known so far. Therefore, it needs to be further researched.

## 4. Conclusion

*Agaricus blazei Murrill,* a mushroom of biomedical importance, contains a number of bioactive components, many of them biological are response modifiers which activate our immune systems for a multitude of defensive functions (Figure 3). Polysaccharides of *A. brasiliensis* have been known to have anticancer, antiviral, and immunomodulatory effects, and other substances are probably involved as well. Moreover, $\beta$-glucans and their enzymatically hydrolyzed oligosaccharides (AO) from *A. brasiliensis* show antihyperglycemic, antihypertriglyceridemic, antihypercholesterolemic, and antiarteriosclerotic activities [25]. Therefore, the pharmacological effects and health function of *A. brasiliensis* are more and more focused on in the world. Although there seems to be clear evidences that ABM extract are rich in $\beta$-glucans, which presumably contribute to the observed pharmacological activities, isolation, and dose response studies, as well as chemical identification and quantification of specific compounds responsible for the potential benefit from ABM, mushroom ingestion should be fully developed. Careful clinical studies comparing the activity of the whole mushroom extracts, isolated compounds, and epidemiological data still need to determine whether *A. brasiliensis* provides real clinical benefits.

## Conflict of Interests

The authors declare that there is no conflict of interests.

## Acknowledgment

This work was supported by the Foundation of Jinan Science and Technology Development Program (201303055).

## References

[1] C. U. Lima, C. O. Cordova, O. D. Nóbrega, S. S. Funghetto, and M. G. Karnikowski, "Does the *Agaricus blazei* Murill mushroom have properties that affect the immune system? an integrative review," *Journal of Medicinal Food*, vol. 14, no. 1-2, pp. 2–8, 2011.

[2] M. L. C. Gonzaga, N. M. P. S. Ricardo, F. Heatley, and S. D. A. Soares, "Isolation and characterization of polysaccharides from *Agaricus blazei* Murill," *Carbohydrate Polymers*, vol. 60, no. 1, pp. 43–49, 2005.

[3] H. Kawagishi, T. Kanao, R. Inagaki et al., "Formolysis of a potent antitumor (1 → 6)-$\beta$-d-glucan-protein complex from *Agaricus blazei* fruiting bodies and antitumor activity of the resulting products," *Carbohydrate Polymers*, vol. 12, no. 4, pp. 393–403, 1990.

[4] C.-H. Shu and B.-J. Wen, "Enhanced shear protection and increased production of an anti-tumor polysaccharide by *Agaricus blazei* in xanthan-supplemented cultures," *Biotechnology Letters*, vol. 25, no. 11, pp. 873–876, 2003.

[5] M. L. Gonzaga, D. P. Bezerra, A. P. Alves et al., "In vivo growth-inhibition of Sarcoma 180 by an $\alpha$-(1 → 4)-glucan-$\beta$-(1 → 6)-glucan-protein complex polysaccharide obtained from *Agaricus blazei* Murill," *Journal of Natural Medicines*, vol. 63, no. 1, pp. 32–40, 2009.

[6] T. Mizuno, T. Hagiwara, T. Nakamura et al., "Antitumor activity and some properties of water-soluble polysaccharides from 'Himematsutake', the fruiting body of *Agaricus blazei* Murill," *Agricultural and Biological Chemistry*, vol. 54, pp. 2889–2896, 1990.

[7] M. Mizuno, K.-I. Minato, H. Ito, M. Kawade, H. Terai, and H. Tsuchida, "Anti-tumor polysaccharide from the mycelium of liquid-cultured *Agaricus blazei* mill," *Biochemistry and Molecular Biology International*, vol. 47, no. 4, pp. 707–714, 1999.

[8] M. Endo, H. Beppu, H. Akiyama et al., "Agaritine purified from *Agaricus blazei* Murrill exerts anti-tumor activity against leukemic cells," *Biochimica et Biophysica Acta*, vol. 1800, no. 7, pp. 669–673, 2010.

[9] H. Akiyama, M. Endo, T. Matsui et al., "Agaritine from *Agaricus blazei* Murrill induces apoptosis in the leukemic cell line U937," *Biochimica et Biophysica Acta*, vol. 1810, no. 5, pp. 519–525, 2011.

[10] T. Takaku, Y. Kimura, and H. Okuda, "Isolation of an antitumor compound from *Agaricus blazei* Murill and its mechanism of action," *Journal of Nutrition*, vol. 131, no. 5, pp. 1409–1413, 2001.

[11] F. Firenzuoli, L. Gori, and G. Lombardo, "The medicinal mushroom *Agaricus blazei* murrill: review of literature and pharmaco-toxicological problems," *Evidence-Based Complementary and Alternative Medicine*, vol. 5, no. 1, pp. 3–15, 2008.

[12] L. C. Faccin, F. Benati, V. P. Rincão et al., "Antiviral activity of aqueous and ethanol extracts and of an isolated polysaccharide from *Agaricus brasiliensis* against poliovirus type 1," *Letters in Applied Microbiology*, vol. 45, no. 1, pp. 24–28, 2007.

[13] F. T. G. de Sousa Cardozo, C. M. Camelini, A. Mascarello et al., "Antiherpetic activity of a sulfated polysaccharide from *Agaricus brasiliensis* mycelia," *Antiviral Research*, vol. 92, no. 1, pp. 108–114, 2011.

[14] M.-F. Wu, Y.-M. Hsu, M.-C. Tang et al., "*Agaricus blazei* Murill extract abrogates $CCl_{14}$-induced liver injury in rats," *In Vivo*, vol. 25, no. 1, pp. 35–40, 2011.

[15] J.-B. Chang, M.-F. Wu, Y.-Y. Yang et al., "Carbon tetrachloride-induced hepatotoxicity and its amelioration by *Agaricus blazei* Murrill extract in a mouse model," *In Vivo*, vol. 25, no. 6, pp. 971–976, 2011.

[16] L. Chen, H. J. Shao, and Y. B. Su, "Coimmunization of *Agaricus blazei* Murill extract with hepatitis B virus core protein through DNA vaccine enhances cellular and humoral immune responses," *International Immunopharmacology*, vol. 4, no. 3, pp. 403–409, 2004.

[17] C.-H. Hsu, K.-C. Hwang, Y.-H. Chiang, and P. Chou, "The mushroom *Agaricus blazei* Murill extract normalizes liver function in patients with chronic hepatitis B," *Journal of Alternative and Complementary Medicine*, vol. 14, no. 3, pp. 299–301, 2008.

[18] B. Grinde, G. Hetland, and E. Johnson, "Effects on gene expression and viral load of a medicinal extract from *Agaricus blazei* in patients with chronic hepatitis C infection," *International Immunopharmacology*, vol. 6, no. 8, pp. 1311–1314, 2006.

[19] H. Inuzuka and T. Yoshida, "Clinical utility of ABCL (*Agalicus Mushroom* Extract) treatment for C-type hepatitis," *Japanese Pharmacology and Therapeutics*, vol. 30, no. 2, pp. 103–107, 2002.

[20] H. Mukai, T. Watanabe, M. Ando, and N. Katsumata, "An alternative medicine, *Agaricus blazei*, may have induced severe

hepatic dysfunction in cancer patients," *Japanese Journal of Clinical Oncology*, vol. 36, no. 12, pp. 808–810, 2006.

[21] V. E. C. Ooi and F. Liu, "Immunomodulation and anti-cancer activity of polysaccharide-protein complexes," *Current Medicinal Chemistry*, vol. 7, no. 7, pp. 715–729, 2000.

[22] M. Mizuno, M. Morimoto, K.-I. Minato, and H. Tsuchida, "Polysaccharides from *Agaricus blazei* stimulate lymphocyte T-cell subsets in mice," *Bioscience, Biotechnology and Biochemistry*, vol. 62, no. 3, pp. 434–437, 1998.

[23] J. G. Lin, M. J. Fan, N. Y. Tang et al., "An extract of *Agaricus blazei* Murill administered orally promotes immune responses in murine leukemia BALB/c mice in vivo," *Integrative Cancer Therapies*, vol. 11, pp. 29–36, 2012.

[24] C. U. J. O. Lima, V. C. Souza, M. C. Morita, M. D. Chiarello, and M. G. de Oliveira Karnikowski, "*Agaricus blazei* Murrill and inflammatory mediators in elderly women: a randomized clinical trial," *Scandinavian Journal of Immunology*, vol. 75, no. 3, pp. 336–341, 2012.

[25] Y.-W. Kim, K.-H. Kim, H.-J. Choi, and D.-S. Lee, "Anti-diabetic activity of $\beta$-glucans and their enzymatically hydrolyzed oligosaccharides from *Agaricus blazei*," *Biotechnology Letters*, vol. 27, no. 7, pp. 483–487, 2005.

[26] T. W. Oh, Y. A. Kim, W. J. Jang et al., "Semipurified fractions from the submerged-culture broth of *Agaricus blazei* Murill reduce blood glucose levels in streptozotocin-induced diabetic rats," *Journal of Agricultural and Food Chemistry*, vol. 58, no. 7, pp. 4113–4119, 2010.

[27] F. C. Di Naso, R. N. de Mello, S. Bona et al., "Effect of *Agaricus blazei* Murill on the pulmonary tissue of animals with streptozotocin-induced diabetes," *Experimental Diabetes Research*, vol. 2010, p. 543926, 2010.

[28] A. Niwa, T. Tajiri, and H. Higashino, "*Ipomoea batatas* and *Agarics blazei* ameliorate diabetic disorders with therapeutic antioxidant potential in streptozotocin-induced diabetic rats," *Journal of Clinical Biochemistry and Nutrition*, vol. 48, no. 3, pp. 194–202, 2011.

[29] D. G. Valadares, M. C. Duarte, J. S. Oliveira et al., "Leishmanicidal activity of the *Agaricus blazei* Murill in different Leishmania species," *Parasitology International*, vol. 60, no. 4, pp. 357–363, 2011.

[30] D. G. Valadares, M. C. Duarte, L. Ramírez et al., "Therapeutic efficacy induced by the oral administration of *Agaricus blazei* Murill against *Leishmania amazonensis*," *Parasitology Research*, vol. 111, pp. 1807–1816, 2012.

[31] D. G. Valadares, M. C. Duarte, L. Ramírez et al., "Prophylactic or therapeutic administration of *Agaricus blazei* Murill is effective in treatment of murine visceral leishmaniasis," *Experimental Parasitology*, vol. 132, no. 2, pp. 228–236, 2012.

[32] S. L. Friedman, "Seminars in medicine of the Beth Isreal Hospital, Boston, the cellular basis of hepatic fibrosis, mechanismas and treatment strategies," *The New England Journal of Medicine*, vol. 328, no. 25, pp. 1828–1835, 1993.

[33] A. M. Gressner, "Cytokines and cellular crosstalk involved in the activation of fat-storing cells," *Journal of Hepatology*, vol. 22, no. 2, pp. 28–36, 1995.

[34] C. S. Lieber, "Prevention and treatment of liver fibrosis based on pathogenesis," *Alcoholism*, vol. 23, no. 5, pp. 944–949, 1999.

[35] W. J. Brattin, E. A. Glende Jr., and R. O. Recknagel, "Pathological mechanisms in carbon tetrachloride hepatotoxicity," *Journal of Free Radicals in Biology and Medicine*, vol. 1, no. 1, pp. 27–38, 1985.

[36] R. A. Boulton, M. R. Alison, M. Golding, C. Selden, and H. J. F. Hodgson, "Augmentation of the early phase of liver regeneration after 70% partial hepatectomy in rats following selective Kupffer cell depletion," *Journal of Hepatology*, vol. 29, no. 2, pp. 271–280, 1998.

[37] H. H. Song, H. S. Chae, S. R. Oh, H. K. Lee, and Y. W. Chin, "Anti-Inflammatory and anti-allergic effect of *Agaricus blazei* extract in bone marrow-derived mast cells," *The American Journal of Chinese Medicine*, vol. 40, no. 5, pp. 1073–1084, 2012.

[38] J. L. Gonçalves, E. H. Roma, A. C. Gomes-Santos et al., "Pro-inflammatory effects of the mushroom *Agaricus blazei* and its consequences on atherosclerosis development," *European Journal of Nutrition*, vol. 51, pp. 927–937, 2012.

[39] Z. Sui, R. Yang, B. Liu et al., "Chemical analysis of *Agaricus blazei* polysaccharides and effect of the polysaccharides on IL-1$\beta$ mRNA expression in skin of burn wound-treated rats," *International Journal of Biological Macromolecules*, vol. 47, no. 2, pp. 155–157, 2010.

[40] T. Watanabe, A. Kawashita, S. Ishi et al., "Antihypertensive effect of $\gamma$-aminobutyric acid-enriched *Agaricus blazei* on mild hypertensive human subjects," *Nippon Shokuhin Kagaku Kogaku Kaishi*, vol. 50, no. 4, pp. 167–173, 2003.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2013, Article ID 985636, 6 pages
http://dx.doi.org/10.1155/2013/985636

*Research Article*

# The Protective Effect of Cordymin, a Peptide Purified from the Medicinal Mushroom *Cordyceps sinensis*, on Diabetic Osteopenia in Alloxan-Induced Diabetic Rats

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Received 17 August 2013; Accepted 23 August 2013

Academic Editor: Bo Cui



The aim of this study was to investigate the protective effect of cordymin on diabetic osteopenia in alloxan-induced diabetic rats and the possible mechanisms involved. The diabetic rats received daily intraperitoneal injection with cordymin (20, 50, and 100 mg/kg/day) for 5 weeks. Cordymin could restore the circulating blood glucose, glycosylated hemoglobin (HbA1c), serum alkaline phosphatase (ALP), tartrate resistant acid phosphatase (TRAP), and insulin levels in a dose-dependent manner. Also, the treatment of diabetic rats with cordymin could partially reverse the $\beta$ cells death and decrease the total antioxidant status (TAOS) in the diabetic rats. The results may directly and indirectly account for the possible mechanism of the beneficial effect of cordymin on diabetic osteopenia, which was confirmed with the increased bone mineral content (BMC) and bone mineral density (BMD) in diabetic rats ($P < 0.05$). All those findings indicate that cordymin may play a protective role in diabetic osteoporosis.

## 1. Introduction

An increasing number of diabetic patients are affected by chronic complications, such as cardiovascular disease, kidney disease, diabetes erectile dysfunction, and diabetic osteoporosis [1–4]. The association between diabetes and decreased BMD has been confirmed in adults (Figure 1), and BMD appears to be decreased in both the spine and hip in diabetic patients [5, 6]. These associations may be related to increased risk of fractures in individuals with diabetes, increased risk of diabetes mellitus in individuals with osteopenia, or both. Growing evidence from clinical studies indicates that osteopenia always leads to an increased incidence of bone fracture and a delay in healing of bone fractures and affects the quality of life in diabetic patients [7]. Therefore, searching for effective drugs which can control the development of diabetic osteopenia is of great significance for patients with diabetic osteopenia.

*Cordyceps sinensis* (CS) has been used as a tonic for longevity, endurance, and vitality for thousands of years by the Chinese. Many studies have shown that CS regulates insulin sensitivity [8] and decreases plasma cholesterol levels [9]. The effect of *Cordyceps sinensis* on osteoporosis had been studied in our former paper [10]. So we herein hypothesize that *Cordyceps sinensis* will be beneficial in preventing osteopenia in diabetes and influencing the longer-term course of glycemic control. We undertook the present study to ascertain if cordymin, a peptide purified from the medicinal mushroom *Cordyceps sinensis*, could be accounted for the putative beneficial effect of CS on diabetic osteopenia in diabetic rats.

## 2. Material and Methods

*2.1. Preparation of Cordymin.* *Cordyceps sinensis* was collected from Qing Hai, China. Cordymin was prepared by the way introduced by Wong et al. [11]. Briefly, dried fruiting bodies of *Cordyceps sinensis* (100 g) were homogenized in liquid nitrogen with a pestle, extracted in distilled water

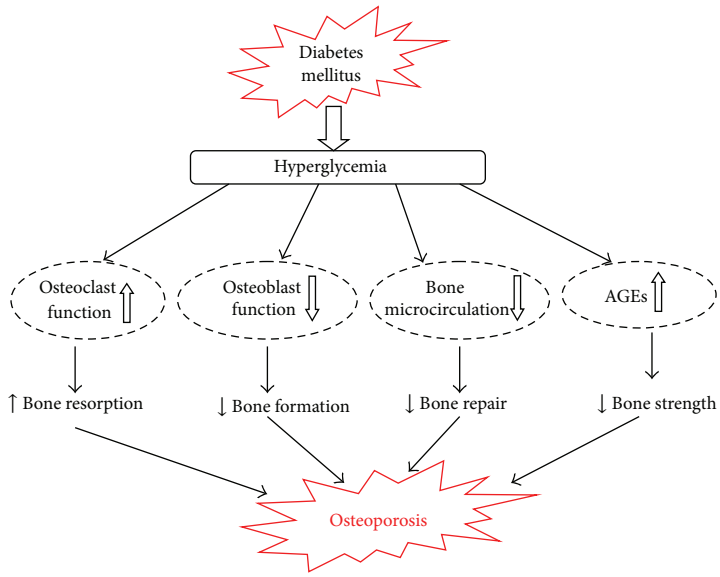



Figure 1: Possible mechanisms through which diabetic conditions increase the risk of osteoporotic fractures (AGEs: advanced glycation end products).

and centrifuged. To the resulting supernatant, ammonium acetate buffer (pH 4.5) was added until a final concentration of 20 mM was attained. The sample was loaded on an SP-Sepharose column. The adsorbed fraction was eluted with 1 M NaCl in 20 mM ammonium acetate buffer (pH 4.5), then dialyzed against distilled water, and lyophilized. Then it was dissolved in 20 mM $NH_4OAc$ buffer (pH 4.5) and applied on a Mono S column and eluted with the same buffer. The fraction containing cordymin was concentrated and then purified on a Superdex 75 column in the same buffer. The single peak eluted constituted purified peptide designated as cordymin.

The molecular mass determination of cordymin was analyzed by means of SDS-PAGE and Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOFMS) in an Applied Biosystems 4700 Proteomics Analyzer.

The N-terminal amino acid sequence of cordymin was analyzed by means of Automated Edman Degradation using a Hewlett Packard 1000A protein sequencer equipped with an HPLC system.

*2.2. Experimental Design.* Thirty Sprague-Dawley male rats were divided into 5 groups ($n = 10$) at random. The rats in the diabetic and cordymin treatment groups were fasted for 10 h and intraperitoneally injected with 75 mg/kg of alloxan to induce diabetes; the remaining 10 control rats were also fasted for 10 h and injected with 0.9% saline. Forty-eight hours after the injection, blood glucose was >16 mmol/L and urine glucose was >+ indicating that diabetes was successfully induced in the rats. The rats in the cordymin treatment group were intraperitoneally injected with cordymin (20, 50, and 100 mg/kg/day) for 5 weeks, and the other 2 groups of rats were intraperitoneally injected with saline (6 mL/kg/day). The body weights of the animals were recorded weekly during the experimental period.

At the end of the experimental period, the animals were fasted overnight (18 h) and then sacrificed by decapitation, and the blood was collected to be centrifuged at 3000 rpm for 20 min, and the clear serum was separated for biochemical analysis. The pancreas was dissected out and placed in 10% buffered formalin, and the liver was dissected out for the measurement of total antioxidant status (TAOS). Femurs were dissected and filled in physiological saline and stored at −20°C for measurement of total bone mineral content (BMC) and bone mineral density (BMD) by Dual-energy X-ray absorptiometry.

*2.3. Biochemical Methodology.* The blood glucose was analyzed with a Glucometer-4 (Bayer) and HbA1c with the HbA1c Apparatus (Variant II, Bio-Rad Laboratories). Serum insulin level was determined with an enzyme-linked immunosorbant assay (ELISA) kit (Nanjing Jiancheng Bioengineering Co. Ltd., China). Serum alkaline phosphatase (ALP) and tartrate resistant acid phosphatase (TRAP) activity were determined by nitrophenol-based method as described by Bessy et al. [12] and Godkar [13], respectively.

*2.4. Estimation of the Damaged Pancreatic β Cells.* The pancreatic tissues were embedded in paraffin blocks after formalin fixation. Paraffin sections were cut at 4 $\mu$m thickness and were deparaffinized in xylene twice for 5 min and then were rehydrated with the graded ethanol. The sections were examined after hematoxylin and eosin (H&E) staining.

*2.5. Estimation of the Total Antioxidant Activity.* The total antioxidant status (TAOS) of hepatic tissue was determined by the way introduced by Laight et al. [14]. The increase in absorbance at 405 nm was measured by using a microplate reader (Shanghai Xunda Medical Technology Inc., China).

*2.6. Estimation of Bone Mineral Content (BMC) and Bone Mineral Density (BMD).* The left femur and L-4 vertebra were mineralized at the temperature of 620°C for 48 h and weighed. The mineralized bones were dissolved in 6 M HCl, and then calcium content in the bone mineral content was assayed by a colorimetric method. BMD was calculated by BMC of the measured area.

## 3. Results

*3.1. Effect of Cordymin on the Body Weights of the Diabetic Osteopenic Rats.* The body weights of the hyperglycemic rats induced by alloxan are presented in Figure 2. The initial body weights of the rats were similar between groups. The body weights of the alloxan-induced diabetic rats were significantly lower than that of the control rats. Contrasted with the diabetic group, the body weights of rats in cordymin-treated group were increased gradually 21 days later ($P < 0.05$, $P < 0.01$).

*3.2. Effect of Cordymin on the Blood Glucose and HbA1c Levels of the Diabetic Osteopenic Rats.* The results of blood glucose from hyperglycemic rats induced by alloxan are presented in Table 1. The serum glucose levels of the saline-treated diabetic rats were significantly higher than that of other rats ($P < 0.05$). Treatment with cordymin at 50 mg/kg and 100 mg/kg significantly lowered the serum glucose level in diabetic rats ($P < 0.05$). Meanwhile, cordymin could decrease the concentration of HbA1c in plasma of alloxan-induced hyperglycemic group 5 weeks later ($P < 0.01$),as shown in Table 1.

*3.3. Effect of Cordymin on Serum Insulin of the Diabetic Osteopenic Rats.* As shown in Table 2, the levels of serum insulin elevated after administration of cordymin100 ($P < 0.05$). However, the same results did not occur in other groups throughout the total duration of the study.

*3.4. Effect of Cordymin on Plasma Enzyme of the Diabetic Osteopenic Rats.* In the present study, significant increase in ALP and TRAP levels, two bone formation markers, was observed in alloxan-induced hyperglycemic group (Table 3). On the contrary, cordymin at 100 mg/kg significantly decreased ALP and TRAP levels ($P < 0.01$) in diabetic rats, while cordymin at 50 mg/kg and 20 mg/kg had little influence on the two bone formation markers in diabetic rats.

*3.5. Effect of Cordymin on the Damaged Pancreatic β Cells.* Histopathological evaluation revealed severe β cells death in the diabetic rats (Figure 3(a)). In contrast, such loss of cells was not seen in the islet cells of the control rats (Figure 3(b)). The β cells of the rats fed with cordymin −100 were partially recovered (Figure 3(c)).

*3.6. Effect of Cordymin on Total Antioxidant Activity of the Diabetic Osteopenic Rats.* We measured TAOS activity as an indirect indication of the formation of $O_2^-$ and other oxidant species. This index was increased in the diabetic groups induced by alloxan in comparison with the control group. In contrast, the TAOS activities of both the cordymin −100 and cordymin −50 groups were lower than those of diabetic group ($P < 0.05$ and $P < 0.01$) (Figure 4).

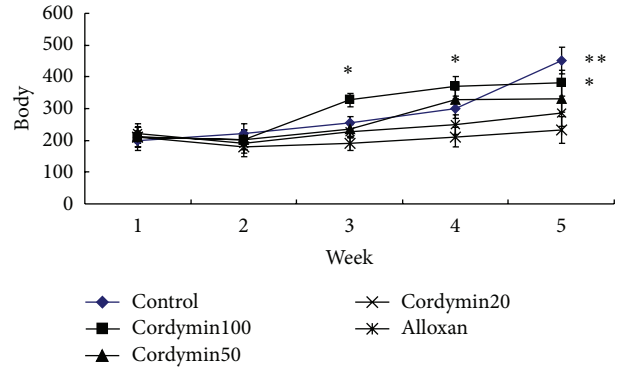



Figure 2: Effects of cordymin on the weekly average body weights. Values are means ± SEM, $n = 10$. *Different from alloxan group $P < 0.05$; **different from alloxan group, $P < 0.01$.

Table 1: Effect of cordymin on blood glucose and HbA1c levels in alloxan-diabetic osteopenic rats.

| Different groups | Blood glucose (mmol/L) | Results of HbA1c (%) |
|---|---|---|
| Alloxan-treated | 21.0 ± 2.0 | 10.8 ± 0.20 |
| Cordymin100-treated | 13.2 ± 3.0* | 8.0 ± 0.30* |
| Cordymin50-treated | 19.2 ± 3.2* | 10.0 ± 0.25 |
| Cordymin20-treated | 18.8 ± 2.5 | 9.8 ± 0.30 |
| Control group | 6.0 ± 1.5 | 4.8 ± 0.21 |

Values are shown as means ± SEM, $n = 10$. *$P < 0.05$ versus alloxan group.

Table 2: Effect of cordymin on serum insulin level in alloxan-diabetic osteopenic rats.

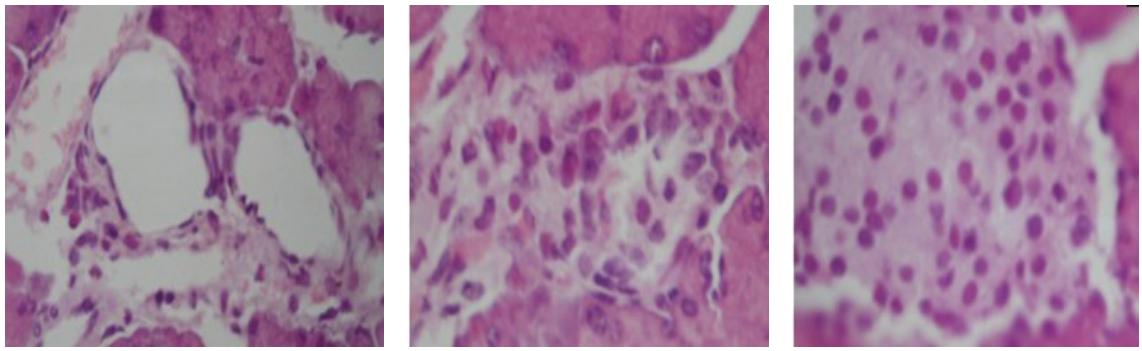
| Different groups | Serum insulin (μU/mL) |
|---|---|
| Alloxan-treated | 4.4 ± 1.1 |
| Cordymin100-treated | 8.4 ± 0.2* |
| Cordymin50-treated | 6.1 ± 1.1 |
| Cordymin20-treated | 5.5 ± 1.0 |
| Control group | 8.5 ± 1.2 |

Values are shown as means ± SEM, $n = 10$. *$P < 0.05$ versus alloxan group.

Table 3: Effects of cordymin on plasma enzymes in alloxan-diabetic osteopenic rats.

| Different groups | TRAP level (uM) | ALP level (mM) |
|---|---|---|
| Alloxan-treated | 0.83 ± 0.11 | 7.20 ± 0.10 |
| Cordymin100-treated | 0.46 ± 0.03* | 4.00 ± 0.03* |
| Cordymin50-treated | 0.50 ± 0.01 | 5.90 ± 0.05 |
| Cordymin20-treated | 0.70 ± 0.05 | 6.30 ± 0.05 |
| Control group | 0.20 ± 0.11* | 3.25 ± 0.12 |

Values are shown as means ± SEM, $n = 10$. *$P < 0.05$ versus alloxan group.



(a) (b) (c)

Figure 3: Islet cell death and replication represented by hematoxylin-eosin. The islet cells of diabetic rats of alloxan treatment (a) showed extensive cell lysis, representing loss of plasma membrane with condensed nuclei and dissolved cytoplasm in wide intercellular spaces. In contrast, the islet cell of cordymin100-fed rat (b) was partly recovered. The islet cell of control rat was (c).

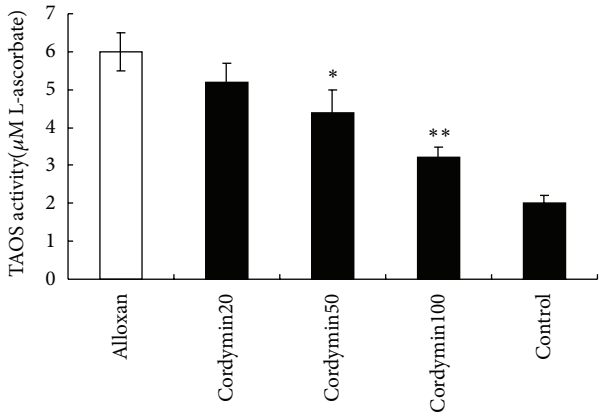



Figure 4: Effect of cordymin on total antioxidant. Activity values are shown as means ± SEM. $^{*}P < 0.05$ versus alloxan group; $^{**}P < 0.01$ versus alloxan group.

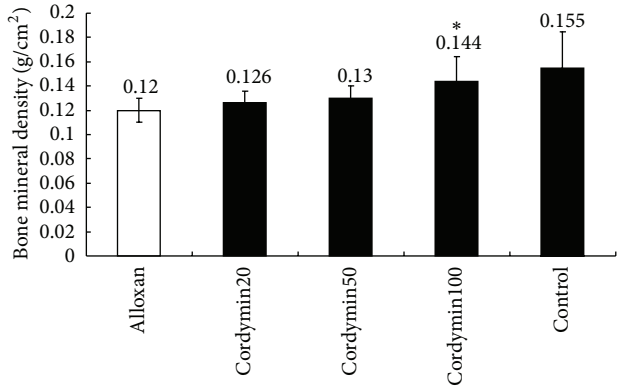



Figure 5: Effect of cordymin on bone mineral density (BMD). Values are shown as means ± SEM. $^{*}P < 0.05$ versus alloxan group.

Table 4: Effects of cordymin on bone mineral content.

| Different groups | Total bone mineral content (mg) | Calcium content (mg/g) |
|---|---|---|
| Control group | $309.05 \pm 8.16^{a}$ | $389.65 \pm 10.22^{a}$ |
| Alloxan-treated | $263.26 \pm 5.63^{b}$ | $326.25 \pm 9.66^{b}$ |
| Cordymin100-treated | $316.11 \pm 6.20^{a}$ | $443.21 \pm 12.23^{a}$ |
| Cordymin50-treated | $316.11 \pm 6.20^{a}$ | $443.21 \pm 12.23^{a}$ |
| Cordymin20-treated | $316.11 \pm 6.20^{a}$ | $443.21 \pm 12.23^{a}$ |

The different letters in the same column indicate a statistical difference ($P < 0.05$).

*3.7. Effect of Cordymin on BMC and BMD.* The BMC in the examined bones of the diabetic groups was significantly reduced compared to the results obtained for the controls. The administration of cordymin100 and cordymin50 to the diabetic animals increased significantly the BMC in the examined bones when compared to the diabetic group (Table 4). Meanwhile, cordymin at 100 mg/kg significantly increased the femur BMD in diabetic rats ($P < 0.05$) (Figure 5).

## 4. Discussion

The wide spread chronic disorder of diabetes mellitus adversely affects multiple organ systems including bones. One of the serious skeletal complications in bones is osteoporotic fractures due to weakened bone strength. The relationship between diabetes and osteoporosis is widely reported [15–17]. Rat models of diabetic osteopenia have contributed significantly to the pathophysiologic understanding of these clinical challenges with regard to bone turnover, bone regeneration, and pharmacologic therapies [18, 19]. However, the animal model of diabetic osteopenia was usually induced with streptozotocin (STZ), and fewer models were induced with an intravenous dose of alloxan. In the present study, we investigated the effects of cordymin on diabetic osteoporosis using the alloxan-induced diabetic rat model.

Alloxan diabetes has been commonly utilized as an animal model of insulin-dependent diabetes mellitus (IDDM). Alloxan produces selective cytotoxicity in pancreatic $\beta$ cells

through the generation of reactive oxygen species resulting in reduced synthesis and release of insulin [20]. In the present study, we examined the antidiabetogenic effect of cordymin by monitoring the $\beta$ cells number, and islet mass was assessed by morphometry on immunostained tissue sections. The $\beta$ cells death and alteration of islet cell population were prominent in the alloxan-induced diabetic rats (Figure 1(a)). In addition, alloxan-induced type 1 diabetes reduced bone quality. Alloxan-treated rats showed a decrease in femoral BMC and BMD compared to normal control rats (Table 4 and Figure 5). It demonstrated the significant association between diabetes and osteoporosis.

The present study was performed to investigate the effectiveness of cordymin on diabetic osteoporosis. Cordymin-treated rats, indeed, exhibited the recovery of $\beta$ cells. It is consistent with the results of cordymin treatment on insulin levels. At the same time, BMC and BMD in diabetic rats treated with cordymin increased when compared to the diabetic rats. Therefore, the beneficial effect of cordymin on pancreatic tissue and its capability of improving serum insulin level might be, at least in part, responsible for the protective effect of cordymin on diabetic osteopenia in the present study.

Hyperglycemia is able to trigger increased oxidative stress, which has been considered to be involved in the pathogenesis of diabetic bone disorders [21]. Cordymin could lower the blood glucose and HbA1c levels, which have been shown to trigger decreased TAOS in diabetic rats. Therefore, the effect of cordymin on diabetic osteopenia in the present study might also be realized indirectly through lowering the concentration of serum glucose, which subsequently triggered a lower extent of oxidative stress in diabetic rats.

Serum alkaline phosphatase (ALP) is a noncollagenous protein secreted by osteoblast, which is essential for bone mineralization [22]. Increased ALP level in serum has been observed in conditions such as rapid bone loss [23] and fracture risk [24, 25]. Tartrate resistant acid phosphatase (TRAP) is secreted by osteoclasts during bone resorption, and serum TRAP activity correlates with resorptive activity in disorders of bone metabolism. In the present study, cordymin significantly decreased ALP and TRAP levels commonly using bone remodeling markers. It suggested that the potency of cordymin is due to decrease of ALP activity and TRAP activity in diabetic rats.

In conclusion, the present study showed for the first time that administration of cordymin has significant effects in rat model of diabetic osteopenia, including dose-dependently restoring the circulating blood glucose, HbA1c, ALP, TRAP, and insulin levels. The beneficial effect of cordymin on diabetic osteopenia might be directly through lowering ALP and TRAP activity and indirectly through recovery of $\beta$ cells and lowering the concentration of serum glucose, which subsequently triggered a lower extent of oxidative stress in diabetic rats.

## Conflict of Interests

The authors declare that there is no conflict of interests.

## Authors' Contribution

Wei Qi and Yang Zhang contributed equally to this work as cofirst authors and Wei Lei, Ya-bo Yan, and Zi-xiang Wu as corresponding authors.

## References


[1] X. Zhang, P. Shi, S. Cao, H. Yu, J. Azad, and S. Ling, "Expression changes of transforming growth factor-betal and thrombospondin-1 in cavernous tissues of diabetic rats," *Urologia Internationalis*, vol. 84, no. 2, pp. 221–225, 2010.

[2] F. J. Del Canizo Gomez, C. Fernandez Perez, I. Moreno Ruiz et al., "Microvascular complications and risk factors in patients with type 2 diabetes," *Endocrinología y Nutrición*, vol. 58, no. 4, pp. 163–168, 2011.

[3] B. Lu, W. Gong, Z. Yang et al., "An evaluation of the diabetic kidney disease definition in chinese patients diagnosed with type 2 diabetes mellitus," *The Journal of International Medical Research*, vol. 37, no. 5, pp. 1493–1500, 2009.

[4] H. Atsumi, M. Kitada, K. Kanasaki, and D. Koya, "Reversal of redox-dependent inhibition of diacylglycerol kinase by antioxidants in mesangial cells exposed to high glucose," *Molecular Medicine Reports*, vol. 4, no. 5, pp. 923–927, 2011.

[5] M. Yamamoto, T. Yamaguchi, M. Yamauchi, H. Kaji, and T. Sugimoto, "Diabetic patients have an increased risk of vertebral fractures independent of BMD or diabetic complications," *Journal of Bone and Mineral Research*, vol. 24, no. 4, pp. 702–709, 2009.

[6] T. Miazgowski, S. Pynka, M. Noworyta-Zietara, B. Kryzanowska-Świniarska, and R. Pikul, "Bone mineral density and hip structural analysis in type 1 diabetic men," *European Journal of Endocrinology*, vol. 156, no. 1, pp. 123–127, 2007.

[7] P. Vestergaard, "Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes-a meta-analysis," *Osteoporosis International*, vol. 18, no. 4, pp. 427–444, 2007.

[8] T. W. Balon, A. P. Jasman, and J. Zhu, "A fermentation product of *Cordyceps sinensis* increases whole-body insulin sensitivity in rats," *Journal of Alternative and Complementary Medicine*, vol. 8, no. 3, pp. 315–323, 2002.

[9] J. H. Koh, J. M. Kim, U. J. Chang, and H. J. Suh, "Hypocholesterolemic effect of hot-water extract from mycelia of *Cordyceps sinensis*," *Biological and Pharmaceutical Bulletin*, vol. 26, no. 1, pp. 84–87, 2003.

[10] W. Qi, Y. B. Yan, P. J. Wang, and W. Lei, "The co-effect of *Cordyceps sinensis* and strontium on osteoporosis in ovariectomized osteopenic rats," *Biological Trace Element Research*, vol. 141, no. 1–3, pp. 216–223, 2010.

[11] J. H. Wong, T. B. Ng, H. Wang et al., "Cordymin, an antifungal peptide from the medicinal fungus Cordyceps militaris," *Phytomedicine*, vol. 18, no. 5, pp. 387–392, 2011.

[12] O. A. Bessy, O. H. Lowry, and M. J. Brock, "A method for the rapid determination of alkaline phosphatase with five cubic millimetres of serum," *Journal of Biological Chemistry*, vol. 164, pp. 321–329, 1946.

[13] P. Godkar, "Enzymes," in *Textbook of Medical Laboratory Techniques*, pp. 149–167, Bhalani Publishing House, Mumbai, India, 1994.

[14] D. W. Laight, P. T. Gunnarsson, A. V. Kaw, E. E. Änggård, and M. J. Carrier, "Physiological microassay of plasma total antioxidant

status in a model of endothelial dysfunction in the rat following experimental oxidant stress in vivo," *Environmental Toxicology and Pharmacology*, vol. 7, no. 1, pp. 27–31, 1999.

[15] N. P. Botushanov and M. M. Orbetzova, "Bone mineral density and fracture risk in patients with type 1 and type 2 diabetes mellitus," *Folia Medica*, vol. 51, no. 4, pp. 12–17, 2009.

[16] P. Vestergaard, L. Rejnmark, and L. Mosekilde, "Diabetes and its complications and their relationship with risk of fractures in type 1 and 2 diabetes," *Calcified Tissue International*, vol. 84, no. 1, pp. 45–55, 2009.

[17] L. A. Ahmed, R. M. Joakimsen, G. K. Berntsen, V. Fønnebø, and H. Schirmer, "Diabetes mellitus and the risk of non-vertebral fractures: the Tromsø study," *Osteoporosis International*, vol. 17, no. 4, pp. 495–500, 2006.

[18] E. A. Thorndike and A. S. Turner, "In search of an animal model for postmenopausal diseases," *Frontiers in Bioscience*, vol. 3, pp. c17–c26, 1998.

[19] B. Portha, O. Blondel, P. Serradas et al., "The rat models of non-insulin dependent diabetes induced by neonatal streptozotocin," *Diabete et Metabolisme*, vol. 15, no. 2, pp. 61–75, 1989.

[20] K. Sakurai, M. Katoh, K. Someno, and Y. Fujimoto, "Apoptosis and mitochondrial damage in INS-1 cells treated with alloxan," *Biological and Pharmaceutical Bulletin*, vol. 24, no. 8, pp. 876–882, 2001.

[21] Y. Hamada, H. Fujii, and M. Fukagawa, "Histomorphometric analysis of diabetic osteopenia in streptozotocin-induced diabetic mice: role of oxidative stress in diabetic bone disorder," *Bone*, vol. 45, supplement 1, pp. S35–S38, 2009.

[22] L. M. Havill, L. G. Hale, D. E. Newman, S. M. Witte, and M. C. Mahaney, "Bone ALP and OC reference standards in adult baboons (Papio hamadryas) by sex and age," *Journal of Medical Primatology*, vol. 35, no. 2, pp. 97–105, 2006.

[23] P. D. Ross and W. Knowlton, "Rapid bone loss is associated with increased levels of biochemical markers," *Journal of Bone and Mineral Research*, vol. 13, no. 2, pp. 297–302, 1998.

[24] G. Patrick, E. Sornay-Rendu, B. Claustrat, and P. D. Delmas, "Biochemical markers of bone turnover, endogenous hormones and the risk of fractures in postmenopausal women: the OFELY study," *Journal of Bone and Mineral Research*, vol. 15, no. 8, pp. 1526–1536, 2000.

[25] P. D. Ross, B. C. Kress, R. E. Parson, R. D. Wasnich, K. A. Armour, and I. A. Mizrahi, "Serum bone alkaline phosphatase and calcaneus bone density predict fractures: a prospective study," *Osteoporosis International*, vol. 11, no. 1, pp. 76–82, 2000.