Antioxidative, anti-inflammation and lung-protective effects of mycelia selenium polysaccharides from *Oudemansiella radicata*

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**A R T I C L E   I N F O**

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**A B S T R A C T**

The present work was designed to investigated the antioxidant, anti-inflammation, and pulmonary protective effects of SMPS and MPS from *Oudemansiella radicata* on LPS-induced lung injured mice. The results demonstrated that SMPS showed potential effects on relieving lung injury and preventing oxidative stress, reflecting by decreasing the serum levels of C3, CRP and GGT, increasing the pulmonary activities of SOD, GSH-Px, CAT and T-AOC, as well as down-regulating the MDA and LPO contents, respectively. Furthermore, the levels of TNF-α (224.211 ± 3.12 ng/mL), IL-1β (254.557 ± 2.18 ng/L), and IL-6 (18.214 ± 0.15 ng/L) in BALF of mice treated with SMPS at the dosage of 400 mg/kg/d significantly lower than that in the lung injured mice. These conclusions indicated that both SMPS and MPS possessed potent antioxidants and anti-inflammation activities, and could be used as functional foods and natural drugs in preventing lung injury.

1. Introduction

The lung injury, which accompanied with the symptoms of diffuse inflammation, aggravated oxidative stress and increased permeability of pulmonary capillaries, has no-eutherapeutic treatment currently, inducing a high mortality clinically [1]. Previous literatures have indicated that the incentives which lead to the lung injury are possibly be trauma, pneumonia, acid aspiration and the bacterial-infected sepsis [2]. Experimentally, the lipopolysaccharide, one of the component of the Gram-negative bacterial cell membrane, is well characterized to establish a lung-injured model for evaluating the therapeutic potential effects of lung diseases [3]. And the lipopolysaccharide can significantly improve the levels of inflammatory cytokines including TNF-α, IL-1β and IL-6, which can aggravate the aggregation of neutrophilic leukocytes, leading the lung injury [4]. In addition, documented researches have proved that the lung-injured is related with the oxidative stress, which is derived from the metabolite of free radicals [5]. However, no effective medical therapies for lung injuries have been published [6]. Thus, it seems to be necessary to find a natural substance with non-toxic on inhibiting the lung injury. *Oudemansiella radicata* which is classified in genus *Oudemansiella* of the family *Tricholomataceae* were widely inhabited on the soil surface or rotten woods located in the broad-leaved forest [7]. Furthermore, as an edible and medicinal mushrooms, many biological-active substances including mucin and oudemansin have been found in *O. radicata* [8]. As an abundant substance from mushrooms, increasing literatures have demonstrated that polysaccharides have become a competitive and hot research recently contributing to the various bioactivities including antioxidant, anti-aging, anticancer, antibacterial, immunomodulatory and anti-inflammationary [9–11]. However, as we are known, present literatures have mainly focused on the polysaccharides from fruiting body, scare article about the antioxidant and lung-protective effects of MPS from the mycelia of *O. radicata* has been published. Meanwhile, the selenium, an essential trace element in the body, is a cofactor of many selenium-dependent enzymes and amino acids such as GSH-Px and selenocysteine [12,13]. And Se-deficiency will

**Abbreviations:** BALF, bronchoalveolar lavage fluid; CAT, catalase; C3, complement 3; LPO, content and lipid peroxide; GC, gas chromatography; GGT, glutamyl transpeptidase; GSH-Px, glutathione peroxidase; hs-CRP, hypersensitive C-reactive protein; IL-1β, interleukin-1 beta; IL-6, interleukin-6; LPS, lipopolysaccharide; MDA, malondialdehyde; MPS, mycelia polysaccharides; SMPS, mycelia selenium polysaccharides; SOD, superoxide dismutase; T-AOC, total antioxidant capacity; TNF-α, tumor necrosis factor-alpha.

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lead to many diseases containing cardiovascular, Keshan and bone diseases [14,15]. Besides, the organic-Se has been proved to show superior biological activities than inorganic ones owing to the nontoxic properties [16]. Recently, the submerged fermentation technique is widely applied in the production of organic-Se due to the short period, high yield and high-purity [17]. Hence, it is quite necessary and significative to explore the SMPS extracted from the mycelia of O. radicata and evaluate its anti-inflammatory capacity in preventing the LPS-induced lung injury.

The purpose of this paper was designed to investigated the antioxidant, anti-inflammatory and lung-protective capacity of the SMPS and MPS, aiming to find the feasibilities of the SMPS and MPS for lung injury. In addition, the monosaccharides components were also analyzed presently.

2. Materials and methods

2.1. Strains and culture conditions

The strain of O. radicata used in present experiment was provide by Shandong Agricultural University (Taian, China). The Se-mycelium was produced by liquid fermentation, which was activated by petridish and then inoculated to the fresh medium of potato 200 g/L, glucose 20 g/L, KH2PO4 1.5 g/L, MgSO4·7H2O 1 g/L and Na2SeO3 2.63 mg/L (liquid medium of the mycelia monosaccharides do not contain Na2SeO3), with natural pH and incubated on a rotary shaker at 120 rpm for 10 days at 25 °C.

2.2. Preparation of SMPS and MPS

The SMPS and MPS were prepared by the method of Liu et al. [18] with slight modifications. Presently, the extracting temperature was 60 °C, and the ethanol precipitation was adopted with triploid ethanol (95%, v/v) overnight (4 °C). The resulting precipitation, obtained by centrifugation (3000 rpm, 15 min) and lyophilization (Beili, Beijing, China and SCIENTZ, Ningbo, China), was considered as SMPS and MPS, respectively.

2.3. Acute toxicity study

Eighteen male Kunming strain mice were collected for the acute toxicity study. Totally, the mice were divided into six groups with three in each group, and the mice were gavaged with SMPS and MPS at increasing dosages of 600, 900 and 1200 mg/kg/d, respectively. The mice were observed continuously for gross behavioral changes, toxic symptoms and mortality during the whole feeding period.

2.4. Animals experiments

The Kunming strain male mice (aging 8–10 weeks and weighing 18–22 g) were provided by Taibang Biological Products Ltd. Co. (Taian, China). The animal experiments were processed according to the National Institute of Health Guide for the Care and Use of Laboratory Animals. All mice were housed under controlled environmental conditions (temperature 22 ± 1 °C, relative humidity 50 ± 5% and a 12 h light/dark cycle), during which time food and water were available ad libitum.

After adaptation for seven days, all mice were randomly divided into six groups (ten in each group) including one normal control (NC) group, one model control (MC) group, and four dosage groups of two high dosage groups (400 mg/kg/d), and two low dosage groups (200 mg/kg/d). Before the polysaccharides intervened, all the mice except in the NC groups were intraperitoneally induced by LPS (5 mg/kg/d) in three successive days, using saline solution as control in NC groups. The experiment was lasted for 30 consecutive days, and at the end of the procedure, all mice were sacrificed by exsanguinations under diethyl ether anesthesia after fasting for 12 h.

The lung tissue was freshly harvested and weight to obtain the wet weight (m1). After keeping warm at 60 °C for 48 h, the lung was weight to obtain the dry weight (m2). The wet-to-dry (W/D) ratio was calculated to assess the degree of tissue edema by the following formula:

\[
W/D\text{ratio} = \frac{m_1}{m_2} 
\]

The collection of BALF was performed three times with 1 ml normal saline (total volume 3 ml). After centrifugation (3000 rpm, 4 °C) for 20 min, the supernatants of the BALF were collected for immunological analysis by ELISA. The TNF-α, IL-1β, and IL-6 were evaluated by commercial kits according to independent instructions.

The lung tissue was immediately homogenized (1:9, w/v) in phosphate buffer solutions (PBS, 0.2 mol/L, pH 7.4). After centrifugation (3000 rpm, 4 °C) for 20 min, the supernate was collected to afford further analysis. The superoxide dismutase activity, glutathione peroxidase activity, catalase activity, total antioxidant capacity activity, malondialdehyde content and lipid peroxide content were evaluated by commercial kits according to independent instructions.

The blood samples obtained from retrobulbar vein were centrifuged at 4000 rpm at 4 °C for 20 min to gain the serum. The glutamyl transpeptidase activity, hypersensitive C-reactive protein, and complement 3 were analyzed by automatic biochemical analyzer (ACE, USA).

2.5. Monosaccharide composition analysis

The monosaccharide compositions were determined by gas chromatography (GC–2010, Shimadzu, Japan) using the previous method [19]. Monosaccharide components were analyzed by comparison with eight monosaccharides including rhamnose, fucose, ribose, arabinose, xylose, mannose, galactose and glucose. The relative molar ratios of monosaccharides were identified by the area normalization method according to the standard chromatograms [20].

2.6. Statistical analysis

All the data were presented as mean ± standard deviation (SD). The differences between groups were analyzed by one-way ANOVA and paired-sample t test (SPSS 16.0 software package, USA). \(P < 0.05\) were considered statistically significant.
3. Results

3.1. Acute toxicity studies

Obviously, no significant changes in behavioral, autonomic and toxic responses, as well as no deaths were observed in the mice treated with SMPS and MPS even at dose of 1200 mg/kg/d, not only at the end of the experiment, but during the investigation period.

3.2. Effects of SMPS and MPS on the degree of lung edema

In order to define the degree of pulmonary edema, the W/D ratio of lung were calculated, and the results were shown in Table 1. The W/D ratio of mice in MC groups was 9.585 ± 0.36%, dramatically higher than that in the NC groups (4.018 ± 0.23%). Interestingly, with the increase of the dose, the lung edema was significantly remitted, demonstrating that oral administration of SMPS and MPS could recovered the LPS-injured lung damage to some extent. Especially in the high-dose group of SMPS (400 mg/kg/d), the W/D ratio reached 4.038 ± 0.28%, which was almost consistent with that in the NC groups.

3.3. Effects of SMPS and MPS on the levels of TNF-α, IL-1β and IL-6 in BALF

Clinically, the levels of inflammatory cytokines TNF-α, IL-1β and IL-6 in the BALF were commonly used as biochemical markers for lung damage. As shown in Fig. 1, the levels of TNF-α, IL-1β and IL-6 in MC group were evidently increased when compared with that in the NC groups (P > 0.0001, P = 0.0006 and P = 0.0012, respectively), indicating that the inflammatory reaction had been appeared in the lung. Interestingly, after the pretreatment with SMPS and MPS at the dose of 400 mg/kg/d, the TNF-α levels reached 224.211 ± 3.52 and 235.621 ± 2.99 ng/mL with the decreasing rates of 15.1 ± 1.33% and 10.8 ± 1.13% (Fig. 1A), the IL-1β levels reached 254.557 ± 2.18 and 263.492 ± 2.41 ng/L with the decreasing rates of 20.7 ± 0.67% and 17.9 ± 0.75% (Fig. 1B), and the IL-6 levels reached 18.214 ± 0.15 and 18.839 ± 0.14 ng/L with the decreasing rates of 24.5 ± 0.62% and 21.9 ± 0.58% when compared with that in MC groups (Fig. 1C), respectively. These results indicated that both SMPS and MPS could markedly suppressed the expression of these cytokines.

3.4. Effects of SMPS and MPS on SOD, GSH-Px, CAT and T-AOC activities

In order to analyze the antioxidant activity of SMPS and MPS in vivo, four kinds of enzymes related to oxidative stress were investigated (SOD, GSH-Px, CAT and T-AOC activities), and the results were described in Fig. 2. Obviously, significant decrease of SOD, GSH-Px, CAT and T-AOC activities were observed after the injection of LPS, indicating that the lung suffered serious oxidative damage. Briefly, the SOD activity of mice treated by SMPS and MPS at the dosage of 400 mg/kg/d reached 159 ± 2.22 and 141 ± 2.7 U/mg protein, with the increasing ratio of 200.0 ± 4.18% and 166.0 ± 5.09% when compared with that in the MC groups, respectively (Fig. 2A, P < 0.0027). As shown in Fig. 2B, the GSH-Px activity of mice treated by SMPS and MPS at 400 mg/kg/d showed obvious recovery effects, which were 241.1 ± 16.7% and 131.2 ± 18.4% higher than that in the MC groups. In Fig. 2C, the CAT activities of mice in the groups treated with SMPS and MPS at the dosage of 400 mg/kg/d were increased to 221.1 ± 4.23% and 196.7 ± 3.49% when compared with that in the MC groups. The non-enzyme activity was illustrated in Fig. 2D, in the dosage groups of 400 mg/kg/d treated with SMPS, the T-AOC activities reached 63.0 ± 2.64 U/mg protein, with 16.67 ± 4.88% higher than that of groups treated with MPS at the dosage of 400 mg/kg (54 ± 2.22 U/mg protein, P = 0.0041) and 215.0 ± 13.2% higher than that of the MC group (20 ± 2.09 U/mg protein, P = 0.0006). Interestingly, all the enzymatic activities of mice in dosage groups treated by SMPS manifested significant increase when compared with that in the dosage groups in MPS,
indicating that the SMPS showed superior effects in enhancing antioxidant systems. Besides, both the group of SMPS and MPS were expressed dose-dependently.

3.5. Effects of SMPS and MPS on MDA and LPO contents

Compared with that in the NC groups, the MDA and LPO contents in LPS-induced lung injured mice (MC groups) were significantly ($P=0.0069$) increased, indicating that the LPS could badly deteriorated the oxidative stress in lung (Fig. 3). The MDA contents in dosage groups treated by SMPS and MPS at 400 mg/kg/d reached $6.4 \pm 0.5$ and $8.31 \pm 0.49$ nmol/mg protein, significantly lower than that in the MC groups, respectively (Fig. 3A). Similarly, the LPO contents in treatment group (SMPS and MPS) were also lower than that in MC groups ($60.7 \pm 2.12\%$ and $54.9 \pm 2.45\%$, Fig. 3B). The results showed that both SMPS and MPS had potential capacity to relieve lung injury by inhibiting the LPO and MDA contents. Furthermore, the SMPS is obviously better than the MPS in suppressing the production of MDA and LPO, which indicating that Se-adding might be enhanced the bioactivity of polysaccharide.

3.6. Effects of SMPS and MPS on the serum biochemical index

As classic serum-indexes on evaluating the lung injury caused by inflammation, the activities of GGT, as well as levels of C3 and hs-CRP were analyzed, and the results were shown in Fig. 4. Obviously, when compared with that in the NC groups, the GGT activities and the levels of C3 and hs-CRP were significantly increased in MC group ($P=0.0013$), indicating that severe inflammation reaction had been occurred in the lung. Briefly, as shown in Fig. 4A, the GGT activities reached $56.3 \pm 1.25$ and $61.7 \pm 1.35$ U/L in the mice treated by SMPS and MPS at the dosage of 400 mg/kg/d, $55.7 \pm 0.98\%$ and

![Figure 2](image2.png)

**Fig. 2.** Effects of SMPS and MPS on the activities of (A) GSH-Px, (B) SOD, (C) CAT, and (D) T-AOC in LPS-induced lung injured mice. The data represented the mean $\pm$ SD of ten mice per group. (a) $P<0.01$ compared with NC group, and (c) $P<0.01$ compared with MC group.

![Figure 3](image3.png)

**Fig. 3.** Effects of SMPS and MPS on the contents of (A) MDA and (B) LPO in LPS-induced lung injured mice. The data represented the mean $\pm$ SD of ten mice per group. (a) $P<0.01$ compared with NC group, and (c) $P<0.01$ compared with MC group.
51.4 ± 1.06% lower than that in the MC groups. Likewise, after treatment with SMPS and MPS at 400 mg/kg/d, the level of C3 decreased by 46.7 ± 1.20% and 53.6 ± 1.32% when compared with that in MC group (Fig. 4B, *P* = 0.0021). Moreover, as illustrated in Fig. 4C, in the high dose of SMPS (400 mg/kg/d), the activities of hs-CRP reached 3.41 ± 0.15 ng/L, which were 70.6 ± 1.29% and 17.6 ± 3.74% lower than that in MC groups and in MPS group at 400 mg/kg/d. The present results indicated that the SMPS showed superior activities than MPS in relieving the lung injury.

3.7. Monosaccharide composition analysis

Comparing the retention time of the standards, the SMPS was composed of mannose, glucose, galactose, and ribose in a percentage composition of 24%, 37.5%, 27.3%, and 11.2% with the molar ratio of 16:21:15:7, while MPS contained mannose, glucose, galactose in a percentage composition of 17.8%, 71.7%, and 10.5% with the molar ratio of 6:20:3 (Table 2).

<table>
<thead>
<tr>
<th>Monosaccharide</th>
<th>SMPS (%)</th>
<th>MPS (%)</th>
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<tbody>
<tr>
<td>Mannose</td>
<td>24.0</td>
<td>17.8</td>
</tr>
<tr>
<td>Glucose</td>
<td>37.5</td>
<td>71.7</td>
</tr>
<tr>
<td>Glucose</td>
<td>27.3</td>
<td>10.5</td>
</tr>
<tr>
<td>Ribose</td>
<td>11.2</td>
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</tbody>
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--- data were not detected in the polysaccharides.

### 4. Discussions

In recent years, the research in polysaccharides have become a hotspot due to its strong and natural immune and antioxidant activities [21]. As for the modification of polysaccharides, a variety of modified methods including sulfation, acetylation, and carboxymethylation have been adopted owing to many advantages such as high stability, safety, superior bioactivity, and non-poisonous [22,23]. Nevertheless, scarce report about the selenylation for polysaccharides extracted from the mycelium of *O. radicata* has been published. Furthermore, the selenium plays important roles on the development and maintenance of a healthy body. However, the contents of selenium in natural food extremely depend on soils, and the selenium deficiencies is still world widely [14]. Hence, it seems necessary to exploit safe resources for Se-supply. Previous literatures had demonstrated that organic selenium is more effective and safer than inorganic ones (such as sodium selenite and selenious acid), and the mushrooms have been regard as natural vectors for selenium accumulations [24,25]. Thus, it is quite significative to explore polysaccharides from the Se-enriched mycelium of *O. radicata* (SMPS) and evaluate the protective effects against the lung injury.

The lipopolysaccharide, an essential component of the Gram-negative bacteria outer membrane, can access the blood stream and initiate the inflammatory reaction, which may develop to shock and even ultimately to death [26]. And the lung injury induced by LPS, characterized by the release of inflammatory cytokines, pneumoniedema, extensive neutrophil infiltration and increased capillary permeability, was supposed to a critical disease syndrome for its high mortality [27]. In this work, the mice with severely lung injury were successfully established induced by LPS injection.

The W/D ratio, which was a typical symptom in both systemic and local inflammation, was clinically evaluated as an index of pulmonary edema [28]. In this study, we had found that the W/D ratio in dosage group had significantly decreased by the interference with SMPS and MPS, indicating that both SMPS and MPS had significant effects in relieving the pulmonary edema. The cytokines of TNF-α, IL-1β and IL-6, as the typical symptom...
of inflammation in alveolar, were significant for evaluating lung inflammation [29]. When lung injury occurred, as the neutrophils aggregation, the pro-inflammatory cytokines including TNF-α, IL-1β and other pro-inflammatory compounds had been released, amplifying the inflammatory response. The Tumor necrosis factor-alpha, which was the original and primary endogenous mediator of the process of an inflammatory reaction, mainly produced by monocytes/macrophages, and could activate the inflammatory cascade, causing damage to the vascular endothelial cells, and inducing alveolar epithelial cells to produce other cellular factors including IL-6 [30,31]. In this study, the remarkable increases of TNF-α, IL-1β and IL-6 levels were found in LPS-induced mice when compared with that in NC group. However, the levels of these cytokines were decreased markedly in BALF after the interference of SMPS and MPS, indicating that the polysaccharide extracted from O. radiata could reduce the content of cytokines on attenuating the lung injury.

Increasing evidence had demonstrated that the oxidized stress damage played vital in the progress of lung injury experimentally and clinically [32–34]. Thus, it quite desired in increasing the antioxidant capacities against the lung injury. Briefly, SOD, GSH-Px and CAT formed the endogenous antioxidant enzyme defenses system, and T-AOC reflected the non-enzymatic antioxidant capacity [35]. These metabolic compounds are important endogenous substances, sustaining the normal physiological functions in vivo, especially against the ROS damage. The mechanism might be that superoxide radicals could be catalyzed by SOD and formed H2O2, which later was decomposed to H2O and O2 by GSH-Px and CAT, thereby the formation of ROS was prevented [36]. The malondialdehyde, a main index of oxidative stress, was the main decomposition product of lipid peroxidation (LPO), and was applied to evaluate the degree of cell damage causing by reactive oxygen metabolites [37,38]. In the present work, significant decrease of SOD, GSH-Px, and CAT activities, as well as remarkably increase of MDA and LPO contents were observed after the injection of LPS when compared with that in the NC groups, indicating that severe oxidative damage had been occurred in lung. Interestingly, when administrated by polysaccharides (SMPS and MPS), significantly changes were showed in dosage groups mice compared to the model control mice. The possible mechanism may be contributing effects of the polysaccharides in activating the enzyme activities and suppressing the progress of lipid peroxidation. Coincidentally, *Termitomyces albuminus* polysaccharides had the similar efficacy in previous studies [39]. The results indicated that the *O. radiata* had potent pulmonary protective effects for providing meaningful therapeutic applications against lung injury.

Serum compositions, containing multiple markers for investigating organic damage, were commonly used as biochemical markers for early lung injuries. According to the previous literatures, increased serum activities of GGT, as well as levels of hs-CRP and C3 were accompanied by the lung damage [40,41]. The C3, as the critical compounds in complement cascade, was an indication of tissue inflammation injury, reflecting the increments of inflammatory factors in LPS-induced lung injury [42]. Besides, the hs-CRP and GGT were also closely associated with inflammation [40,43]. The results of this work revealed that the significant reductions in GGT, C3, and hs-CRP were observed in the lung injured mice after the administration of SMPS and MPS, suggesting the pulmonary recovery of these polysaccharides.

Documented literatures had demonstrated that bioactivities of polysaccharides were mainly associated with monosaccharide compositions [44]. In the present work, the monosaccharide compositions suggested that the major component in both SMPS and MPS were glucose. The same result was expressed in *Pleurotus eryngii* polysaccharides [44]. Furthermore, only ribose was found in SMPS, indicating that the ribose might play vital roles in maintaining the antioxidant and anti-inflammatory status.

5. Conclusions

In the present study, SMPS and MPS were successfully obtained from the mycelium of *O. radiata*, and the antioxidant abilities, pulmonary prevention effects as well as monosaccharide compositions were investigated. The results showed that both SMPS and MPS had potential antioxidant activities and impressive protective effects against LPS-induced lung injury. Furthermore, the special monosaccharide composition ribose was first discovered in SMPS. These results indicated that both SMPS and MPS had potential abilities in the prevention and alleviation of lung damage and its complications.

Competing interests

The authors declared that they had no competing interests.

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