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Characterization, anti-oxidation and anti-inflammation of polysaccharides by *Hypsizygus marmoreus* against LPS-induced toxicity on lung

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ABSTRACT

In our present work, the polysaccharides (MPS) were successfully extracted from the mycelia of *Hypsizygus marmoreus*, and purpose of our work was to evaluate the analysis of the characterization, anti-inflammation and anti-oxidation against the lung failure induced by lipopolysaccharide (LPS) in mice. Pretreatment with MPS at 800 mg/kg markedly ameliorated the lung wet-to-dry weight (W/D) ratio and pulmonary histopathological conditions, reduced the tumor necrosis factor-alpha (TNF)-α, interleukin (IL)-6, and IL-1β levels in bronchoalveolar lavage fluid (BALF), myeloperoxidase (MPO) activities. In addition, the protective effects of MPS might be attributed to the down-regulations of the serum complement 3 (C3), glutamyl transpeptidase (GGT) and high-sensitivity C-reactive protein (hs-CPR) contents, as well as improving the antioxidant status by enhancing pulmonary enzyme activities (catalase (CAT), superoxide dismutase(SOD), glutathione peroxidase (GSH-Px) and total antioxidant capacity (T-AOC)) and eliminating the lipid peroxidation (LPO) and malondialdehyde (MDA), respectively. What's more, our work investigated the characterization of the MPS.

1. Introduction

In recent years, extensive attentions have been paid to the natural materials owning to their interesting properties and potential clinical values [1]. Considering the plenty of side-effects and toxicity of synthetic compounds that included many clinically useful therapeutic drugs, people begin to focus more on the natural medicine [2]. The reducing toxicity replacement of chemical compounds by natural sources is important because natural sources are green reagents, eco-friendly and low-toxicity [2,3]. Therefore, much attentions have been focused on natural materials, such as the novel nanomaterials including $FbFe_{12}O_{19}$ and $CuFe_{12}O_{19}$ [3–5]. Meanwhile, the traditional medicinal and edible mushrooms are wide fields for searching new drugs and biological active compounds with superior properties.

Mushroom, traditionally natural used in many countries as a medicine with anti-inflammatory and anti-oxidative properties, is widely used because of its non-toxic and non-side effects. Previous studies indicated that mushrooms have expressed important effects on antioxidation and anti-inflammation, like immunomodulatory properties of *Ganoderma Iucidum* [6], inflammation inhibitory effect of *Trametes pubescens* [7]. Some of these health-promoting properties have been attributed to the polysaccharides produced by different varieties mushrooms, as reported like that, the antioxidant and immunostimulating properties of a polysaccharide isolated from *Cordiceps militaris* [8], and the antioxidation and anti-aging activities of polysaccharide from *Agrocybe cylindracea* [9].

Hypsizigus marmoreus, as a usually useful and popular edible mushroom, also draws the world attention due to its medicinal properties [10]. During the past years, dozens of clinical examinations based on somewhat different strategies have shown that polysaccharides from *H. marmoreus* expressed potential biological activities. However, there are seldom pharmacology researches about the antiflammation effects against the lung damage by polysaccharides from *H. marmoreus*.

Abbreviation: BALF, bronchoalveolar lavage fluid; CAT, catalase; C3, complement 3; DMSO, dimethyl sulfoside side; FT-IR, Fourier transform infrared spectral; GC, gas chromatography; GGT, glutamyl transpeptidase; GSH-Px, glutathione peroxidase; H&E, hematoxylin and esosin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; HPLC, high performance thin layer chromatography; Mn, high performance thin layer Number-average molecular weight; hs-CRP, high-sensitivity C-reactive protein; -OH, hydroxyl groups; IL-1β, Interleukin-1β; IL-6, interleukin-6; LPO, lipid peroxidation; LPS, lipopolysaccharide; MDA, malondialdehyde; MC, model control; MPS, mycelia polysaccharides; MPO, myeloperoxidase; NC, normal control; NMR, nuclear magnetic resonance; PC, positive control; PDA, potato dextrose agar; ROS, reactive oxygen species; SEM, scanning electron microscopy; SD, standard deviation; SOD, superoxide dismutase; T-AOC, total antioxidant capacity; TC, total cholesterol; TG, triglyceride; TNF-α, tumor necrosis factor-alpha; VLDL-C, very low-density lipoprotein cholesterol; Mw, weight-average molecular weight; W/D, wet-to-dry weight; Mz, Z-average molecular weight.

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Lung injury, characterized by the pulmonary edema, has led to high mortality rates worldwide [11]. These inflammatory diseases triggered by kinds of pathogenic factors, and the most common pathogenic factor that plays a determinant role in resulting in lung failure is attributable to lipopolysaccharide (LPS), the major structural element of gramnegative bacteria outer membranes that causes experimental endotoxemia and organ dysfunction [12,13]. LPS can trigger the inflammatory reactions in the process of lung failure by activating macrophages to secrete pro-inflammatory cytokines, including tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6 [14]. Hence, the excessive production of these cytokines led to the immunological disorder, tissue injury and organ dysfunction [15]. Furthermore, LPS is responsible for the synthesis of reactive oxygen species (ROS), which overproduction may lead to a considerable oxidative stress, combining with the increase of malondialdehyde (MDA) as well as the reduction in endogenous antioxidant defenses [16]. Now clinical pharmacological treatment of lung failure is based on new romuscular blocking agents and has been demonstrated to decrease mortality. However, continuous use of synthetic corticosteroid drugs is associated with side-effects and toxicity with long-term use in the clinic, such as hypertension in elderly patients, and stunted growth in children [17], highlighting the urgent requirement for novel natural treatment strategies and medicine.

Consequently, one water-soluble polysaccharide (MPS) from the mycelia from *H. marmoreus* was extracted and prepared for this work. The protective effects on the lungs and the antioxidant activities of MPS in LPS-induced model mice were investigated. In addition, polysaccharide characterization, including monosaccharide compositions, molecular weights and bond types were characterized.

2. Methods and materials

2.1. Chemicals and reagents

The stain of *H. marmoreus* used in this experiment was from our laboratory. The LPS and standard monosaccharides samples were purchased from Sigma Chemicals Co., Ltd. (St. Louis, USA). The diagnostic kits for antioxidant indicators and ELISA analysis were purchased from Nanjing Jiancheng Bioengineering Ins. (Nanjing, China). All other chemicals used in our present experiment were purchased from the Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China).

2.2. Stain and culture

A stain of *H.marmoreus* used in this experiment was from our laboratory and maintained on potato dextrose agar (PDA) slant. The mycelia were produced by the liquid fermentation in PDA culture medium (glucose 20 g/L, KH_2PO_4 1 g/L, $MgSO_4$ 1 g/L and $ZnSO_4$ 1.5 g/L in 1 L H_2O). The liquid culture was grown at 25 °C for 10 d.

2.3. Preparation of MPS

The mycelia of *H. marmoreus* were harvested from the liquid medium by filtration, dried (55 °C) and then shattered into powder. The mycelia powder was extracted three times with distilled water (pH 8.0, 85 °C, 3 h), and then centrifuged at 5000 rpm for 10 min. The supernatants were concentrated, mixed with ethanol (3 v/v, 85%), and then kept at 4 °C for 24 h. After the centrifugation (5000 rpm, 10 min), the precipitate was collected and dried (55 °C). The crude polysaccharide was purified by Sevag method [18], and then the purified polysaccharide was lyophilized.

2.4. Polysaccharide characterization

2.4.1. Scanning electron microscope (SEM) analysis

Field emission scanning electron microscope (S-4800, FE-SEM, Hitachi High-Technologies, Japan) was applied to observe the surface characterization of MPS. The accelerating voltage was 10 kV and the image magnification was of 2,500 ×.

2.4.2. Fourier transform infrared spectral (FT-IR) analysis

The MPS was mixed with KBr powder and then pressed into pellets for infrared spectral analysis within a range of 4000–400 cm^{-1}. The IR spectrum of the polysaccharide was measured by a fourier transform infrared spectrophotometer (Bruker, Germany).

2.4.3. Molecular weight analysis

Molecular weights were analyzed by a high performance thin layer chromatography (HPLC) system (Shimadzu LC-2010AT, Japan) equipped with an Atlantis C18 column (250 mm × 4.6 mm × 5 μm) and a refractive index detector. Deionized water as the mobile phase was at a flow rate of 1 mL/min, and the column temperature was kept at 30 °C. A series of standard dextran solutions were used to generate the calibration curve. And then the Agilent GPC software was applied to define the molecular weights of MPS.

2.4.4. Determination of monosaccharide composition

Monosaccharide composition was determined by gas chromatography (GC) equipped with an Rtx-1 capillary column (30 mm × 0.25 mm × 0.25 μm) according to the reported method [19]. Composition identification was performed by comparison with standard monosaccharides. The relative molar ratios were calculated utilizing the area normalization method according to the chromatogram.

2.4.5. ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy (NMR) analysis

^{13}C and ^{1}H NMR spectroscopy experiments were taken using a 700-MHz Varian Mercury 2010 Magneto Oxford spectrometer at 60 °C, and the sample was dissolved in dimethyl sulfoside side (DMSO).

2.5. Acute toxicity study

The acute toxicity study was done utilizing the method reported by Chao et al. [20]. Ten Kunming strain mice were randomly divided into two groups of five animals each. In the control group, mice were given liberal access to food and water. In the experimental group, mice were given the polysaccharide samples *per os* at a dose of 4000 mg/kg. The creatures were observed continuously for any mortality and gross behavioral changes, including soreness, restlessness, respiratory distress, abnormal locomotion, catalepsy and toxic symptoms.

2.6. Animals experiment

The Kunming stain mice (weighted 20 ± 2 g) were purchased from Taibang Biological Products Ltd. Co. (Taian, China). All the mice were housed in stainless steel cages under controlled conditions (temperature 23 ± 2 °C, lights on 12 h every day). The experiments were performed as approved by the Institutional Animal Care and Use Committee of Shandong Agricultural University, and in accordance with the Animals (Scientific Procedures) Act 1986 (amended 2013).

After the accommodation, all mice were randomly divided into six groups ($n = 10$), the normal control group (NC), the model control group (MC), the positive control group (PC, dexamethasone 2 mg/kg) and three polysaccharides treatment groups (including MPS at the dosage of 200 mg/kg, 400 mg/kg and 800 mg/kg). Before the oral administration of polysaccharides and dexamethasone, the mice were intraperitoneally induced by LPS (5 mg/kg) for three days, except the NC mice which were treated with saline solution only. 30 days after the administration, all mice were sacrificed by diethyl ether asphyxiation.

Blood samples from the orbital sinus were centrifuged (14,000 rpm, 4 °C, 10 min) to yield the required serums. The levels of total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-C), very low density lipoprotein cholesterol (VLDL-C), and low density