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Anti-hyperlipidemic and antioxidant effects of alkali-extractable mycelia polysaccharides by *Pleurotus eryngii* var. *tuolensis*

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ABSTRACT

In this study, we noted that the Al-MPS from Pleurotus eryngii var. tuolensis provoked pharmacological effects on blood lipid profiles and oxidative stress. Animal studies demonstrated that Al-MPS showed potential effects on relieving hyperlipidemia and preventing oxidative stress, reflecting by decreasing the levels of serum enzyme activities (ALP, ALT and AST), restoring the activities of antioxidant enzymes (SOD, GSH-Px, CAT and T-AOC), down-regulating the MDA and LPO contents, as well as remitting the hepatic and cardiac tissues injury, respectively. The serum levels of TC, TG, LDL-C, VLDL-C, and HDL-C on mice treated with Al-MPS (500 mg/kg bw) reached 2.48 ± 0.08, 1.24 ± 0.03, 0.84 ± 0.02, 0.34 ± 0.02, and 1.80 ± 0.03 mmol/L, which were lower/higher against the hyperlipidemia mice. The results clearly indicated that the Al-MPS could be used as a beneficial health food and potentially natural candidate medicine in preventing the high-fat emulsion-induced hyperlipidemia.

1. Introduction

The hyperlipidemia, a disorder of lipid metabolism clinically involving in abnormal serum levels of lipids (Kimoon & Seungho, 2013; Zhang et al., 2013), and widely induced by the high-fat emulsion in many literatures (Wang et al., 2015; Zhao et al., 2012), plays vital roles on the incidence and pathogenesis of diseases including fatty liver and cardiovascular diseases (Ruchel et al., 2016; Zhao et al., 2012). And previous scientific literatures have shown that these diseases are closely related to the ultra-production of reactive oxygen species (ROS) (Liang et al., 2011). In addition, the oxidative damage could accelerate the pathogenic progress of hyperlipidemia and its complications (Li et al., 2010). Clinically, a number of synthetic hypolipidemic drugs are available and effective in the treatment with hyperlipidemia, but the associated adverse effects including diarrhea, nausea, myositis and abnormal liver function severely handicap the applications (Bidkar, Ghanwat, Bhujbal, & Dama, 2013; Wu et al., 2016; Zhang et al., 2013). Natural antioxidants have shown potential effects in scavenging the free radicals, aiming to protect the human body from oxidative stress (Kumar, Ahmed, Gupta, Anwar, & Mujeeb, 2013). Increasing evidences have suggested that the hepatoprotective effects of substance may be linked to their known antioxidant and pre-oxidant properties. Taken together, it is eagerly to develop biologically active components possessing the function of antioxidant and hepatoprotection from natural materials as a better alternative to allopathic drugs and hence to prevent associated toxicity.

Recently, mushrooms have become extremely popular as a dietary nutritious food in Asia owing to the major roles of prevention and treatment of various human diseases (Chen, Ju, Li, & Yu, 2011). The fungi of *Pleurotus eryngii* var. *tuolensis*, known as Bailingu oyster mushroom in China, contains dietary fiber, valuable nutrients, and abundant biological compounds (Cui et al., 2015). And previous reports have focused on the polysaccharides extracted form fruiting bodies of *P. eryngii* var. *tuolensis* (Cha et al.,

Abbreviation: ALP, alkaline phosphatase; ALT, alamine aminotransferase; Ara, arabinose; AST, aspertate aminotransferase; CAT, catalase; Al-MPS, alkali-extractable mycelia polysaccharides; GC, gas chromatography; GSH-Px, GSH peroxide; HDL-C, high-density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; LPO, lipid peroxidation; MC, model control; MDA, malondialdehyde; NC, normal control; PC, positive control; prot, protein; ROS, reactive oxygen species; SOD, superoxide dismutase; T-AOC, total antioxidant capacity; TC, total cholesterol; TG, triglyceride; VLDL-C, very low-density lipoprotein cholesterol.

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2012; Miyazawa, Okazaki, & Ohga, 2008; Yan, Jing, & Wang, 2015). Scare literature about the mycelium has been published. Owing to the advantages of submerged fermentation attributing to shorter incubation time, more-compact culture space, higher production, and lesser chance of contamination availability of convenient control, the mycelia have become an alternative source of mushroom polysaccharides (Gan, Ma, Jiang, Wang, & Zeng, 2012; Wasser, 2011).

Currently, increasing evidences have indicated that the hot water extract of the *P. eryngii* var. *tuolensis* fruiting body show potential cardiac protection against ischemia-reperfusion injury (Cui et al., 2014; Cui et al., 2015; Wang et al., 2014; Yan, Jing, & Wang, 2015). Nevertheless, the residue was usually abandoned. Previous literatures have demonstrated that additional polysaccharides can be isolated in these abandoned residues by alkali solutions (Yu, Yang, Cui, Wang, & Ren, 2014). Besides, a novel alkali-extractable polysaccharide from *P. eryngii* var. *tuolensis* has been reported to show the inhibition effects on the proliferation and apoptosis of human hepatic cancer cells (Cui et al., 2016). To date, little attention has been devoted to the Al-MPS from *P. eryngii* var. *tuolensis*. Gather up the threads, it is therefore quite necessary and significative to explore alkali-extractable polysaccharides from the mycelia of *P. eryngii* var. *tuolensis* in preventing hyperlipidemia induced by the high-fat emulsion.

This study was designed to investigate the antihyperlipidemic and antioxidant activities of Al-MPS from *P. eryngii* var. *tuolensis* on high-fat emulsion-induced hyperlipidaemic mice for seeking clinical antihyperlipidemic mechanisms in pharmaceutical industry. In addition, monosaccharide compositions and structural characterizations of Al-MPS were also investigated.

2. Materials and methods

2.1. Strain and chemicals

The strain of *P. eryngii* var. *tuolensis* was obtained and identified by Fungi Institute of Academy of Agricultural Sciences (Taian, China). The diagnostic kits for investigating SOD activity, CAT activity, GSH-Px activity, T-AOC activity, LPO contents, and MDA contents were purchased from Nanjing Jiancheng Bioengineering Insititute (Nanjing, China). Standard monosaccharide samples were purchased from Sigma Chemicals Company (St. Louis, USA). All other chemicals used in present work were analytically grade and provided by local chemical suppliers in China.

2.2. Extraction and purification of Al-MPS

The liquid fermentation technology was used to produce the mycelia of *P. eryngii* var. *tuolensis* by the liquid medium of glucose (20 g/L), potato (200 g/L), KH_2PO_4 (1.5 g/L), and $MgSO_4$ (1 g/L). After one week of fermentation, the dried mycelia were mixed with proper volumes of sodium hydroxide (1 mol/L), and kept warm at 40 °C for 6 h, and the supernatant liquids were precipitated with ethanol (1:3, v/v) at 4 °C overnight. After centrifugation (3000 × g, 10 min), the precipitate was deproteinated by employing the Sevage method (Miao et al., 2013), purification by loading onto a DEAE-52 cellulose anion-exchange column (1.6 cm × 20 cm) eluting with NaCl solutions (1 mol/L) at a flow of 5 mL/tube, as well as dialysis against deionized water (Baskar & Sathya, 2011). After lyophilization, the precipitate was considered as Al-MPS, and the final polysaccharides yields was 4.73 ± 0.44%.

2.3. Animal experiments

According to the previous report (Wang et al., 2015), the high-fat emulsion contained oil phase of 10 g cholesterol, 25 g liquid lard oil, 25 mL of tween-80, and 1 g methylthiouracil, as well as water phase of 30 mL distilled water, 20 mL propylene glycol, and 2 g sodium deoxycholate were initially prepared. The water phase and oil phase were freshly blended before the animal experiments.

Sixty male mice (Kunming strain) weighing 20 ± 2 g were purchased from Taibang Biological Products Ltd. Co. (Taian, China). All mice were acclimated for 7 days under conditions of temperature (22 ± 2 °C), humidity (55 ± 5%) and a 12 h light-dark cycle, during which time they had free access to standard chow and tap water *ad libitum*. 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 adaption of one week, all mice were randomly allocated into six groups (ten in each group) including three dosage groups of Al-MPS (500, 300, and 100 mg/kg bw), one NC group, one MC group, and one PC group. During the experiment procedure, the gavage of high-fat emulsion and polysaccharides in dosage groups were processed every other day, using distilled water in NC and MC groups, and simvastatin (200 mg/kg bw) in PC groups as controls. The whole experiment was lasted for 40 consecutive days.

At the end of the experiment, the mice were weighted, kept overnight fasting, and sacrificed by exsanguinations under diethyl ether anesthesia. The serum was obtained from the blood by centrifugation at 12,000 × *g* for 10 min. The ALP activities, ALT activities, AST activities, TC levels, TG levels, HDL-C levels, LDL-C levels, and VLDL-C levels in serum were measured using automatic biochemical analyzer (ACE, USA).

The liver and heart were excised, homogenized (1:9, g/mL) in phosphate buffer solutions (PBS, 0.2 mol/L, pH 7.4) immediately. After centrifuging (5000 × g, 4 °C) for 20 min, the supernatants were collected to conduct further research. The activities of SOD, GSH-Px, CAT, and T-AOC, as well as contents of MDA and LPO were determined by the commercial reagent kits according to the instructions.

Fresh liver and heart tissue masses were fixed in 4% formaldehyde solution overnight, embedded in paraffin, cut in slices, and stained with hematoxylin and eosin. The slices were photo-graphed under microscope showing the histopathological changes (× 400 magnifications).

2.4. Monosaccharide composition analysis

Monosaccharide compositions was analyzed by gas chromatography (GC, GC-2010, Shimadzu, Japan) equipped with a capillary column of Rtx-1 (30 m × 0.32 mm × 0.2 μm) using the published method (Sheng, Yu, Xin, Zhao, Zhu, & Hu, 2007). Composition identification was processed by comparison with the standard chromatograms of rhamnose, fucose, ribose, arabinose, xylose, mannose, galactose and glucose, and the relative molar ratios were calculated by the area normalization method.

2.5. Fourier-transform infrared (FT-IR) spectroscopy analysis

The Al-MPS (1 mg) was mixed with KBr powder (100–200 mg) and then pressed into pellets for infrared spectral analysis. The IR spectra were recorded by an infrared spectrometer (Nicolet 6700, Thermo Fisher Scientific, USA) with a range of 4000–500 cm^{-1}.

2.6. ^1H and ^{13}C nuclear magnetic resonance spectroscopy (NMR) analysis

^1H and ^{13}C NMR measurements were conducted using a Bruker AV-300 spectrometer operating at 300 MHz at 25 °C, and the sample was dissolved in deuterated water (D_2O).