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Polysaccharides from *Dendrobium huoshanense* stems alleviates lung inflammation in cigarette smoke-induced mice

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ABSTRACT

The present work investigated the inhibitory activity of the polysaccharide from cultivated *Dendrobium huoshanense* (cDHP) on lung inflammation in cigarette smoke (CS)-induced mouse model. cDHP was mainly composed of mannose and glucose in a molar ratio of 1.89: 1.00, and had a backbone with linkages of 1,4-Man*p*, 1,4-Glc*p*, 1,4,6-Man*p* and 1-Glc*p*. Hematoxylin and Eosin (HE) staining and immunohistochemistry analysis showed that cDHP can increase alveolar number, thicken alveolar wall, inhibit pulmonary bulla formation and decrease inflammatory cell infiltration as compared to the model group. ELISA determination revealed that cDHP can inhibit CS-induced enhancement in TNF-α and IL-1β secretion in serum and lung. These results suggested that cDHP can resist CS-induced lung inflammation. Further, the phosphorylation analysis of p65, IκB, p38 and JNK as well as the DNA binding activity analysis of NF-κB and AP-1 implied that the anti-inflammation function of cDHP is mediated via regulating NF-κB and MAPK signaling.

1. Introduction

Smoking has an indisputable threat to human health (Samet, 2013). Although aggressive efforts have been performed to prevent smoking, there are still about one billion smokers all over the world and the world's annual death toll from smoking have reached about six million (World Health Organization, 2015). It has been accepted that smoking is the major risk factor for pulmonary diseases, heart disease, diabetes mellitus, stroke, cancer, and other systemic related diseases (National Center for Chronic Disease Prevention and Health Promotion Office on Smoking and Health, 2014). For the cigarette smoke-triggered lung diseases, the pulmonary inflammation has been considered as a central pathological basis. Therefore, to find a therapeutic agent resisting pulmonary inflammation is beneficial to the prevention of smoke-induced lung diseases. Recently, there is a considerable interest in identifying preventive factors that may delay the onset or progression of these diseases, and diet therapy has been proved to be a practical approach.

Dendrobium huoshanense C.Z. Tang et S.J., a well-known edible Orchid plant, has been recorded as a traditional Chinese medicine to improve eyesight, cure throat inflammation, treat fever, nourish stomach and regulate lung function for centuries (Bao, Shun, & Chen, 2001; Zha, Deng et al., 2017; Zha, Zhang et al., 2017). *D. huoshanense* has been successfully cultivated in China and the polysaccharides as its main active compounds have been suggested to be capable of exerting a variety of healthy functions including resisting cataract, enhancing immunity, decreasing blood glucose, protecting liver and inhibiting tumor (Li et al., 2015; Tian, Zha, Pan, & Luo, 2013; Xie et al., 2017). Based on our previous works, which found that the polysaccharides from *D. huoshanense* could attenuate the inflammatory aggravation of ethanol-induced and carbon tetrachloride (CCl_4)-induced liver injury in mice (Tian, Zha, & Luo, 2015; Wang, Luo, Chen, Zha, & Wang, 2014) and prevent the inflammatory response of pancreatic tissues in alloxan-induced diabetic mice (Pan et al., 2014), we speculated that *D. huoshanense* polysaccharides might also have beneficial effects to alleviate smoke-induced lung inflammation. Thus, the present work aimed at investigating the potential and possible mechanism of *D. huoshanense* polysaccharides to resist lung inflammation induced by cigarette smoke in mice.

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2. Materials and methods

2.1. Plant materials and reagents

The plants from seeds of wild *D. huoshanense*, which were collected from Dabie Mountain area, China, were cultivated in Huoshan county of Dabie Mountain area and collected in December of 2015. The D-mannose (Man), D-glucose (Glc), Dimethyl sulphoxide (DMSO) and methyl iodide (CH_3I) were purchased from Sigma-Aldrich (MO, USA). The *N*-(3-Dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC), trifluoroacetic acid (TFA), and sodium borodeuteride ($NaBD_4$) were obtained from Aladdin Industrial Corporation (Shanghai, China). Bovine serum albumin (BSA) was purchased from Sangon Biotech (Shanghai, China). ELISA kits for the determination of TNF-α and IL-1β were from Raybiotech Inc (GA, USA). Antibodies for SAPK/JNK, phosphor-SAPK/JNK, p38, phosphor-p38, NF-κB 65, phosphor-NF-κB p65, IκB, phosphor-IκB and β-actin as well as horseradish peroxidase-conjugated goat anti-rabbit IgG were purchased from Cell Signaling Technology (Beverly, MA, USA). All other reagents were analytical grade.

2.2. Extraction of D. huoshanense polysaccharides

One hundred grams of dried *D. huoshanense* stem powders were extracted with four litres 95% (v/v) ethanol for about 12 h. The residues, which were collected and dried at room temperature (RT), were further extracted twice with double distilled H_2O for 2 h in a water bath at 100 °C. The supernatants, after concentrated in a vacuum rotary evaporator under reduced pressure at 60 °C, were mixed with ethanol at a final concentration of 80% (v/v) and stayed overnight at RT for 24 h to precipitate the biomacromolecules. Then, the precipitates were treated with Sevag reagent (Sevag, Lackman, & Smolens, 1938) to remove proteins followed by dialysis with molecular weight cutoff of 3500 Da and lyophilization to give the refined polysaccharide (cDHP), with an average yield of 11.25 ± 2.73%.

2.3. Measurement of physicochemical properties of cDHP

The total carbohydrate content was determined by phenol-sulfuric acid method (DuBois, Gilles, Hamilton, Rebers, & Smith, 1956). The soluble protein content was determined by Bradford method (Hammond & Kruger, 1988). The *O*-acetyl group content was determined by back-titration method (Wei et al., 2016). The intrinsic viscosity [η] was determined by one point method using Ubbelohde viscometer (Pamies, Cifre, Martinez, & de la Torre, 2008). The ash and moisture contents were determined according to the reference (Meyer & Palmer, 1936). UV scanning was performed to analyze whether the existence of proteins and nucleic acids in cDHP.

2.4. Fourier transform infrared (FTIR) spectroscopy analysis

The cDHP was analyzed by FTIR spectroscopy using a Thermo Nicolet 67 instrument (Thermo Electron, Madison, WI, USA). The sample was ground with KBr powder and then pressed into pellets for FTIR scanning in the frequency range of 4000–400 cm^{-1}.

2.5. Monosaccharide composition analysis

According to our previous report (Xie, Liu et al., 2016), the dried cDHP (20 mg) was successively hydrolyzed by 2 M TFA at 110 °C for 4 h, reduced with $NaBH_4$ at RT for 3 h, and acetylated with pyridine and acetic anhydride (1:1, v:v) at 110 °C for 1 h. The alditol acetate derivative was dissolved in methenyl trichloride and analyzed by gas chromatography (7890A GC system, Agilent Technologies, USA). The standard monosaccharide was treated and measured using the methods as same as the above demonstration.

2.6. Methylation analysis

According to the modified Hakomori method (Hu et al., 2017; Wang, Luo, & Zha, 2010), the dried cDHP was dissolved in DMSO, followed by the addition of SMSM (Sodium methylsulfinyl methide) reagent, treatment with an ultrasonic wave at RT for 0.5 h and keeping in the dark overnight. Then, the methyl iodide was added to the mixture to initiate methylation reaction. All the above procedures were performed under a nitrogen flow condition. The methylated product was monitored by FTIR until the stretching band of −OH ranging from 3200 to 3700 cm^{-1} disappeared. After formic acid were added to the dried methylated products at 100 °C for 3 h, the sample was successively hydrolyzed with TFA, reduced with $NaBD_4$, and acetylated with pyridine and acetic anhydride, followed by the analysis on a 7890A-5975C GC–MS system (Agilent Technologies, USA).

2.7. Animals

Fifty male KM mice (6-week old, 18 ± 2 g) with the animal certificate No. 201718631 were purchased from Cavens Lab Animals Co., Ltd. (Changzhou, China). The mice were maintained under specific pathogen-free conditions with a 12/12 h light-dark cycle at 25 ± 2 °C and 40% relative humidity. All animal handling procedures were performed strictly in accordance with the P.R. China Legislation on the Use and Care of Laboratory Animals and were approved by the Animal Care Review Committee, Hefei University of Technology.

2.8. Experimental procedure

After an acclimatization period of one week in the SPF grade animal room, mice were randomly divided into five groups (10 mice per group), including normal group, model group, cDHP low-dose group, cDHP middle-dose group, and cDHP high-dose group. Based on the recommended dietary allowance (6–12 g dry stems for one healthy adult with an average body weight of 60 kg) from Chinese Pharmacopoeia (2015), the extraction yield of cDHP (11.25%) and its designed dose that is equivalent to 5, 10 and 10-fold adult intake per day, the dosages of cDHP used for animal experiment were between 56.25–112.5, 112.5–225.0 and 225.0–450.0 mg/kg/day. Thus, the low-dose, middle-dose and high-dose of cDHP were set at 100 mg/kg/day, 200 mg/kg/day and 400 mg/kg/day, respectively, and were intragastrically administrated to mice for successive 4 weeks. The mice of normal group and model group were intragastrically administrated with an equal volume of normal saline as cDHP treatment for the same period. From the fifteenth day, except the normal group, all the other four groups mice were exposed to cigarette smoke from 4 cigarettes for 1 h per day for two weeks in a closed smoking chamber (80 × 70 × 50 cm) after intragastric administration according to the reported method (Chu et al., 2016; Chen et al., 2010). The commercial, filtered cigarettes (Huangshan brand, 83 mm in length), with 11 mg tar, 1.2 mg nicotine and 11 mg carbon monoxide per cigarette, were produced by China Tobacco Anhui Industrial Co., Ltd. After the last administration, all mice were fasted for 12 h and sacrificed by carbon dioxide asphyxiation. Thereafter, the body weight was measured and blood was collected to separate serum. Meanwhile, the lung, spleen, kidney and liver were removed from the mice and weighted to calculate the organ indexes.

2.9. Lung pathological examination

The lung tissues were fixed in 4% paraformaldehyde at 4 °C overnight and embedded in paraffin. From the paraffin blocks, three micrometer sections were prepared and stained with hematoxylin and eosin (H&E). The histological changes were observed and photographed under a microscope at × 200 magnification. The alveolar size was assessed by the mean lining interval (MLI) (Sato et al., 2007). The