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Effect of an inulin-type fructan from *Platycodon grandiflorum* on the intestinal microbiota in rats exposed to PM2.5

Shan Shan [a,b], Yi Xiong [a,b,1], Jianguo Guo [d,1], Mengyao Liu [a,b], Xin Gao [a,b], Xinjing Fu [d], Deyong Zeng [a,b], Chen Song [a,b], Yingchun Zhang [a], Dayodu Cheng [a], Weihong Lu [a,b,c,*]

[a] School of Chemistry and Chemical Engineering, Harbin Institute of Technology, Harbin, China
[b] National and Local Joint Engineering Laboratory for Synthesis, Transformation and Separation of Extreme Environmental Nutrients, Harbin, China
[c] Institute of Extreme Environment Nutrition and Protection, Harbin Institute of Technology, Harbin, China
[d] Key Laboratory of Human Diseases Animal Model, State Administration of Traditional Chinese Medicine, Beijing 100021, China

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ABSTRACT

In this study, an inulin-type fructan (PGPI-1-a) was isolated from the roots of *Platycodon grandiflorum*. PGPI-1-a was composed of (2 → 1)-linked β-D-fructofuranose (Fruf) and a terminal α-D-glucopyranose (Glcp) with a molecular weight of 12.1 kDa. PM2.5 exposure has brought a great threat to human health in recent years. Therefore, this study explored the effect of PGPI-1-a on the intestinal microbial community structure of rats exposed to PM2.5 using the animal model of PM2.5 inhalation exposure. The results showed that PGPI-1-a could regulate the intestinal microbiota by partly restoring the perturbed levels of *Peptoniphilaceae_[G-2]* and *Lachnospiraceae_[G-2]* caused by PM2.5 exposure. In addition, the relative abundance of *Butyrivibrio*, a butyric acid-producing genera, significantly increased after PGPI-1-a intervention. These results indicated that PGPI-1-a could improve the imbalance of intestinal microbiota due to PM2.5 exposure to a certain extent.

1. Introduction

Platycodon grandiflorum is a perennial herb belonging to the Campanulacea family, mainly found in China, Japan, Korea and other Northeast Asia regions (Xu, Dong, Qiu, Cong, & Ding, 2010). The roots of *Platycodon grandiflorum* are edible and are often used for making pickles in Northeast China. It is also a kind of traditional Chinese medicine material and has been used to treat illnesses such as cough, asthma and lung abscess for many years (Zhang et al., 2015). Polysaccharide is one of the major bioactive compounds in *Platycodon grandiflorum*. It has attracted much attention due to its various pharmacological activities such as immune stimulation (Zheng et al., 2017), anti-angiogenesis (Xu, Dong, Qiu, Ma, & Ding, 2011) and anti-oxidation (Sheng, Liu, Wang, Lv, & Du, 2017), et al.

Inulin, an important storage polysaccharide in many higher plants, are reported to exist in dicotyledonous species such as Asteraceae and Campanulaceae, and monocotyledonous species such as Liliaceae and Poaceae (Xu et al., 2016). Recent studies have shown that inulin-type fructans possessed a variety of beneficial functions, such as anti-inflammatory, anticancer and immunomodulatory activities (Li, Zhang, Cao, Ji, & Gao, 2018). In addition, inulin-type fructans also possess prebiotic activity, which can selectively promote the growth of beneficial bacteria, regulate the composition of intestinal microbiota, thereby improve intestinal health (Fu et al., 2018; Wilson & Whelan, 2017). Pang et al. (2019) obtained inulin-type fructan (PGF) with a degree of polymerization (DP) of 10 from *Platycodon grandiflorum*, and found that PGF could promote the proliferation of six strains of lactobacillus and showed good prebiotic activity. In recent years, many studies have been conducted on the structure of *Platycodon grandiflorum* polysaccharides, but there is almost no relative report on the separation of inulin with higher DP from *Platycodon grandiflorum* to date (Xu et al., 2011; Y. Xu et al., 2010; Zheng et al., 2017).

Haze poses a great challenge to the human living environment. It not only brings many inconveniences to people's lives, but also threatens human health. Fine particulate matter (PM2.5) is reported to be the primary pollutant in haze days, and the levels of PM2.5 are usually higher during winter and heating season (November to March) (Xu & Zhang, 2020; Zhao et al., 2015). The composition of PM2.5 is very

* Corresponding author at: Institute of Extreme Environment Nutrition and Protection, Harbin Institute of Technology, 92# Xidazhijie Street; Harbin, Heilongjiang 150001, China.
E-mail address: lwh@hit.edu.cn (W. Lu).
[1] The co-second author.

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complex, containing heavy metals, elemental carbon, organic carbon, water-soluble ions, bacteria and viruses, et al. (Pang et al., 2021; Zhao et al., 2015). It was reported that PM2.5 exposure may increase the risk of various diseases, including respiratory diseases, neurological diseases, gastrointestinal diseases, cardiovascular diseases, and metabolic diseases, which may be related to the imbalance of intestinal microbiota (Liu et al., 2021; Shou et al., 2019). Therefore, maintaining the steady-state balance of the intestinal microbiota is very essential to human health. Mutlu et al. (2018) reported that PM2.5 exposure significantly increased the alpha diversity of small intestine, colon and feces, and the change seemed to be most apparent in the fecal environment. Liu et al. (2021) found that intratracheal instillation of PM2.5 remarkably changed the diversity and composition of the intestinal microbiota of mice. Wang et al. found that hydroxytyrosol could restore the microbial architecture of mice exposed to PM2.5 to the normal state (Wang et al., 2019). However, there are few reports on the regulatory effect of polysaccharides on the intestinal microbiota of rats exposed to PM2.5 to date.

In this study, a polysaccharide fraction (PGPI-1-a) was obtained from the roots of *Platycodon grandiflorum* through extraction by hot water and purification on DEAE-52 and Sephadex G100 columns. The physicochemical and structural properties of PGPI-1-a was characterized through the combination of various chemical and instrumental methods. In addition, the effect of PGPI-1-a on the intestinal microbiota of rats exposed to PM2.5 was investigated by 16S rRNA sequencing.

2. Materials and methods

2.1. Materials and reagents

The roots (medicinal slices) of *Platycodon grandiflorum* were provided by Heilongjiang University of Chinese Medicine. Sephadex G-100 and DEAE-52 were obtained from SUMMUS Company (Harbin, China). All other reagents were produced in China and analytical grade unless otherwise indicated.

2.2. Preparation of PGPI-1-a

The crude polysaccharide of *Platycodon grandiflorum* (PGP) was obtained according to the method previously described by our group (Shan et al., 2021). The root of *Platycodon grandiflorum* was ground into powder (60 mesh), which was then extracted with 4-fold volume of ethanol (95%, v/v) three times at 80 °C (each time for 2 h) to defat and remove some pigments and small molecules. The dried ethanol-extracted residue was extracted twice with 25-fold volume of distilled water under reflux at 90 °C (each time for 2 h). After filtering and centrifuging, the supernatant was concentrated followed by deproteinization, decolorization, dialysis (cut off, 3500 Da) and drying to give crude polysaccharides (PGP). 25 mg of PGP was dissolved in 500 mL of distilled water, and then 95% ethanol (v/v) was added slowly until the ethanol concentration reached 40% (v/v). The mixture was stirred thoroughly and kept in a 4 °C fridge for 12 h. Then the mixture was centrifuged and the resulting precipitate was lyophilized to give PGPI. Subsequently, PGPI (10 mg/mL) was eluted with distilled water on the DEAE-52 ion-exchange column (2.6 cm × 20 cm) at a flow rate of 1 mL/min. The elution fractions (5 mL/tube) were automatically collected using a fraction collector. The elution curves of PGPI was obtained by measuring the polysaccharide content in each tube using phenol-sulfuric acid method (Chen, Cheong, Song, Shi, & Huang, 2013). The eluted fraction was concentrated and lyophilized to obtain PGPI-1. PGPI-1 was further purified on a Sephadex G-100 gel filtration column (1.6 cm × 60 cm) and eluted with distilled water at a flow rate of 0.8 mL/min to give PGPI-1-a.

2.3. Physicochemical of PGPI-1-a

The polysaccharide content and protein content of PGPI-1-a were determined by phenol-sulfuric acid method and Bradford method, with glucose and bovine serum albumin as standard samples, respectively. The presence of protein or nucleic acid in PGPI-1-a was detected by ultraviolet spectra, whose scanning range was from 190 to 400 nm.

The homogeneity and average molecular weight of PGPI-1-a were determined by the HPGPC system (Jahanbin, Abbasian, & Ahang, 2017). The sample solution (1 mg/mL, 50 μL) was loaded on the Ultrahydrogel™ linear column (7.8 mm × 300 mm, Waters, Japan), and then eluted with phosphate buffer (pH = 6.8) at a flow rate of 0.6 mL/min. The molecular weight of PGPI-1-a was calculated using a dextran standard curve made from dextran standards of different known molecular weights (0.4, 0.7, 1.5, 3, 8 and 12.9 kDa).

The monosaccharide composition of PGPI-1-a was determined by two methods (GC–MS and HPLC) due to the existence of a large amount of unexpected fructose residues in PGPI-1-a. Gas chromatography-mass spectrometry (GC–MS) analysis was completely conducted in accordance with our previous method (Shan et al., 2021). High performance liquid chromatography (HPLC) analysis was carried out according to the methods reported by Xu et al. (2016), with slight modification. 10 mg of the polysaccharide sample was hydrolyzed with TFA (0.1 mol/L) at 105 °C for 1 h. The hydrolysate was neutralized with NaOH solution, and then filtered with a 0.45 μm membrane. Subsequently, the resulting hydrolysate was loaded on the Agilent ZORBAX NH_2 column (250 mm × 4.6 mm, 5 μm) and analyzed by HPLC system (UltiMate 3000, Thermo Fisher Scientific, USA) equipped with an RID detector. The conditions were as follows: the mobile phase was acetonitrile-water (70:30, v/v), the temperature of the column was 35 °C.

2.4. NMR analysis

20 mg of PGPI-1-a was dissolved in 0.5 mL D_2O, and then lyophilized. The above process was repeated three times. The resulting sample was re-dissolved in 0.5 mL D_2O, and then transferred to an NMR tube. After that, the 1D (^{13}C and ^{1}H) and 2D (HSQC, COSY, HMBC and TOCSY) spectra of PGPI-1-a were determined by a 600 MHz NMR spectrometer (Bruker Corp., Switzerland).

2.5. X-ray diffraction

The X-ray pattern of PGPI-1-a was determined on an X-ray diffractometer (XRD, PANalytical, Netherlands) under the following test conditions: Cu Kα radiation (40 kV and 40 mA), scattering angle range (2θ) of 5–80°, and step size of 0.026°. According to XRD patterns, the crystallinity of PGPI-1-a was calculated by Jade 6.0 software.

2.6. Animals and experimental design

The animal experiments were carried out under the National Guidelines for Care and Use of Laboratory Animals. Six-week-old male Brown-Norway (BN) rats were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). After one week of acclimatization in the laboratory, all animals were randomly divided into three groups ($n = 5$): control group (namely group C: normal BN rats plus filtered air), PM2.5 exposure group (namely group B: normal BN rat plus PM2.5) and PGPI-1-a group (namely group BD: normal BN rat plus PM2.5 plus PGPI-1-a). The rats in PM2.5 exposure group were subjected to whole-body exposure using PM2.5 exposure system (Beijing Huironghe Technology Co., Ltd., Beijing,China), while the rats in PGPI-1-a group were intragastrically administered with PGPI-1-a (56 mg/220 g BW) at the end of PM2.5 exposure. The exposure site was located on the first floor of the Institute of Laboratory Animal Sciences, CAMS&PUMC. The experiment was conducted during the heating period in Beijing (December 6, 2017-April 11, 2018) with exposure for 4 h per day