Carbohydrate Polymers 101 (2014) 533–543

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Protective effect of *Millettia pulchra* polysaccharide on cognitive impairment induced by D-galactose in mice

Xing Lin[a,1], Zhongshi Huang[b,1], Xiaoyu Chen[a], Yanping Rong[a], Shijun Zhang[a], Yang Jiao[a], Quanfang Huang[a], Renbin Huang[a,*]

[a] Guangxi Medical University, Nanning, Guangxi 530021, China
[b] Youjiang Medical College for Nationalities, Baise, Guangxi 533000, China

ARTICLE INFO

Article history:
Received 26 May 2013
Received in revised form 7 August 2013
Accepted 14 September 2013
Available online xxx

Keywords:
Polysaccharide
Millettia pulchra
Cognitive impairment
D-Galactose

ABSTRACT

A polysaccharide (PMP) was isolated from *Millettia pulchra* and purified by DEAE-cellulose and Sephadex G-75 chromatography. The results showed that PMP was composed of D-glucose and D-arabinose in a molar ratio of 90.79% and 9.21%, with an average molecular weight of about 14,301 Da. Furthermore, the effect of PMP on cognitive impairment induced by D-galactose in mice was evaluated. Treatment with PMP significantly reversed D-galactose-induced learning and memory impairments, as measured by behavioral tests. One of the potential mechanisms of this action was to reduce oxidative stress and suppress inflammatory responses. Furthermore, our results also showed that PMP markedly reduced the content and deposition of β-amyloid peptide, improved the dysfunction of synaptic plasticity, increased the levels of acetylcholine, but decreased cholinesterase activity. These results suggest that PMP exerts an effective protection against D-galactose-induced cognitive impairment, and PMP may be a major bioactive ingredient in *M. pulchra*.

1. Introduction

Memory decline is characteristic of aging and age-related neurodegenerative disorders which lead to a progressive loss of cognitive function, especially in spatial memory (Zhang, Jiang, Li, Hao, & An, 2007). It has been proposed that formation of reactive oxygen species (ROS) is an important step leading to neuronal death in a variety of age-related neurodegenerative disorders (Castegna et al., 2002; De Iuliis, Grigoletto, Recchia, Giusti, & Arslan, 2005). ROS oxidizes various biological macromolecules, thereby disturbing homeostatics within the neuron and ultimately resulting in cell death. D-Galactose (D-gal) can cause the accumulation of ROS, or stimulate free radical production indirectly by the formation of advanced glycation endprodcts (AGE) in vivo, finally resulting in oxidative stress (Zhang, Li, Cui, & Zuo, 2005). D-Gal can also impair neurogenesis in the dentate gyrus, a process similar to natural aging in mice (Cui et al., 2006). Therefore, D-gal-induced senescent mouse is a good model for studying age-related damage in brain (Wei et al., 2005).

Drugs isolated from traditional medicinal plants may provide a promising therapy on brain injuries caused by oxidative stress. An example of a traditional herb that is often used in popular folk medicine in the Guangxi Zhuang Autonomous Region of China is *Millettia pulchra* (Leguminosae *M. pulchra* Kurz), which is often used as an anti-aging and memory improving agent (Huang, Jiao, Zhang, & Huang, 2008). The active components of *M. pulchra* primarily consist of polysaccharides, amino acids, glycosides and flavonoids (Li, 2011). Our previous studies, which examined the crude polysaccharides content from *M. pulchra* (Huang et al., 2009), demonstrated that treatment with the *M. pulchra* crude polysaccharides significantly increased neurotransmitter content in the brain, promoted the metabolism of free radicals, and improved cognitive abilities in animal dementia models (Huang, Lin, et al., 2008; Huang, Xie, et al., 2008). Furthermore, the crude polysaccharides significantly reduced the over-expression of β-amyloid (Aβ), β-site amyloid precursor protein cleaving enzyme (BACE) and β-amyloid precursor protein (APP) in senescence-accelerated mouse prone 8 mice in a dose-dependent manner (Huang et al., 2009). However, the active ingredients in the crude polysaccharides content and the mechanisms by which these active ingredients ameliorate cognitive deterioration remain elusive. Extracting the bioactive fractions from the crude polysaccharides is a valid approach, which allows for the pharmacological assessment of substrates and effects. Preliminary screening of several fractions from the crude polysaccharides indicated that a polysaccharide named PMP, with composed of arabinosyl and glucosyl residue, may be an active fraction; this fraction showed good neuroprotective action and was a relatively clean ingredient.

* Corresponding author. Tel.: +86 0771 5339805.
E-mail address: huangrenbin518@163.com (R. Huang).
[1] These authors contributed equally to this work.

0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.09.037

Based on the preliminary screening, we hypothesized that PMP could protect the brain from cognitive impairment. In the present study, we investigated the effect of PMP on cognitive impairment and oxidative injury induced by D-galactose in mice. The molecular mechanisms involved in the prevention of learning and spatial memory loss were also studied.

2. Materials and methods

2.1. Drugs and chemicals

M. pulchra (Leguminosae *M. pulchra* Kurz) was purchased from Nanning Qianjinzi Chinese Pharmaceutical Co. Ltd. (Nanning, China). A voucher specimen (YLS20110312619) was identified by Professor Quanfang Huang in the Department of Pharmacognosy, The First Affiliated Hospital of Guangxi University of Chinese Medicine and deposited in the herbarium of Department of Pharmacology of Guangxi Medical University.

D-Galactose (D-gal) was purchased from Sino-American Biotechnology Co., Ltd. (Luoyang, China), and dissolved in physiological saline. Huperzine A was purchased from Yuzhong Drug Manufactory (Henan, China). Malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH), nitric oxide (NO), glutathione peroxidase (GPX), neuronal nitric oxide synthase (nNOS) and total NOS kits were obtained from Nanjing Jiancheng Bioengineering Research Institute (Nanjing, China).

2.2. Preparation of PMP

2.2.1. Preparation of crude polysaccharides from M. pulchra

Dry, sliced *M. pulchra* (1.5 kg) was extracted with 12 L of water for 2 h at 95 °C three times. The combined extracts were filtered, concentrated and centrifuged, and then the supernatant was treated with 3 volumes of ethanol at 4 °C overnight. The precipitate was separated by filtration, washed exhaustively with 95% ethanol, and then dissolved in deionized water and dialyzed using cellulose sacks (8000–10,000 D, Sigma) against flowing water for 48 h. The non-dialyzed portion was precipitated again with three volumes of ethanol overnight. The resulting precipitate was filtrated and washed exhaustively first with anhydrous ethanol, then with acetone and finally with anhydrous ether; 102.86 g of crude polysaccharides was obtained.

2.2.2. Anion-exchange chromatographic method

The crude polysaccharides (100 mg) were dissolved in distilled water and filtered through a membrane (0.45 μm, Nucleopore). Then the solution was applied to a DEAE-Sepharose fast-flow column (3 cm × 45 cm) pre-equilibrated with 20 mM Tris–HCl buffer (pH 7.8). Fractions were prepared in a stepwise elution with increased concentration of NaCl (0.1–1 M) solution at a flow rate of 1.0 ml/min, and with collection of 5 ml for each tube. The polysaccharide content in each fraction was detected by phenol–sulphuric acid method (Zhang et al., 2007). The appropriate fractions were concentrated, dialyzed against water, and finally lyophilized.

2.2.3. Gel-filtration chromatography

Size-exclusion chromatography of the purified polysaccharide was performed on Sephadex G-75 (20 × 600 mm), and 0.1 M NaCl was used as eluant. The major polysaccharides fractions were collected with a fraction collector, then dialyzed with water, and lyophilized to give a polysaccharide named PMP, which was used in subsequent analyses.

2.2.4. Physicochemical property of PMP

Total carbohydrate content of PMP was determined by phenol-sulfuric acid colorimetric method using D-glucose as standard (Taylor, 1995). Protein content was quantified according to the method of Bradford (1976), using bovine serum albumin as standard reference material. Sulphate content was measured from about 10 mg of samples after 2 mol/l HCl hydrolysis (2 h at 100 °C), as described elsewhere (Kawai, Seno, & Anno, 1969). Uronic acid content was estimated by m-hydroxydiphenyl assay using glucuronic acid as the standard (Filisetti-Cozzi & Carpita, 1991).

2.2.5. Molecular weight determination

The homogeneity and molecular weight of PMP were determined by high-performance gel-permeation chromatography (HPGPC). The sample solution (20 μl of 0.5%) was applied to Agilent 1100 HPLC system equipped with a Dhpak SB-803 HQ (8.0 mm × 300 mm), eluted with 0.05 M Na_2SO_4 solution at a flow rate of 0.5 ml/min and detected by a RID-10A refractive index detector. The columns were calibrated with T-series dextran (T-200, T-70, T-40, T-20 and T-10) and glucose as standards. The molecular weight of PMP was estimated by the calibration curve.

2.2.6. Polysaccharide hydrolysis with acid and monosaccharides derivative

The PMP (10 mg) was hydrolysed with 2 M trifluoroacetic acid (TFA) (10 ml) for 8 h at 100 °C in a sealed glass tube. The excess acid was completely removed at 70 °C by a steady stream of nitrogen, and the hydrolysed products were used for the preparation of its derivative. The reaction was carried out by mixing 10 mg of hydrolysed products and 5 ml 0.5 M NaOH, and then 6 ml 0.5 M 1-phenyl-3-methyl-5-pyrazolone methanol solution were added followed by incubation at 70 °C water bath for 30 min. Subsequently the tube was cooled down to room temperature, and 0.5 M HCl was added to neutralize solution pH to 7. Finally, the solution was extracted with 20 ml $CHCl_3$ twice. The extract was centrifuged (3000 rpm, 15 min), the supernatant was collected and the derivatives of the sugars were performed on HPCE. As references, the following neutral sugars were derived and analyzed: L-rhamnose, D-arabinose, D-xylose, D-glucose and D-galactose.

2.2.7. High-performance capillary electrophoresis (HPCE) analysis

HPCE was performed on an uncoated fused-silica capillary tube (50 μm × 70 cm) at 25 °C using 75 mM sodium tetraborate buffer (pH 9.5) as solvent, the absorbance was detected at 254 nm. Before each run, the capillary tube was washed by methanol (5 min), double distilled water (2 min), 0.1 M HCl (5 min), double distilled water (2 min), 0.1 M NaOH (10 min), double distilled water (2 min), and conditioned with the operating buffer for 10 min. The samples to be analyzed were injected automatically, using the low pressure injection mode (0.5 psi), in which the sample is pressurized for 5 s. The injection volume can be calculated with the Poiseuille equation, as proposed by the manufacturer, giving an estimated volume of 6 nl/s of injection time. Electrophoresis was performed at 15 kV using normal polarity. Peak areas were recorded and calculated using the Beckman software system.

2.3. Animals and treatment

Male Kunming mice, weighing 30 ± 2 g, were provided by the Experimental Animal Center of Guangxi Medical University (Guangxi, China). The research was conducted according to protocols approved by the institutional ethical committee of Guangxi Medical University (approval no.: 20110501202). All mice were housed under controlled conditions at 25 ± 2 °C, with a relative humidity of 60 ± 10%, room air changes 12–18 times/h, and a 12-h light/dark cycle. Feed and water were made available ad libitum.

After one week acclimatization, the mice were randomly divided into six groups, each consisting of 15 animals. The six groups were