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Preliminary characterizations, antioxidant and hepatoprotective activity of polysaccharide from *Cistanche deserticola*

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ABSTRACT

To research the preliminary characterizations, antioxidant and hepatoprotective activity of polysaccharides from *Cistanche deserticola* (CDPs), three polysaccharide fractions, CDP-A, CDP-B and CDP-C, were obtained by successively membrane filtration (microfiltration, ultrafiltration and nanofiltration). Molecular weights, monosaccharide compositions, purities and IR spectra of the three fractions were analyzed. Results showed that CDP-C contained higher proportion of galacturonic acid (GalUA) than CDP-B and CDP-A. Antioxidant activities were also analyzed and the results revealed that CDP-C possessed the highest activity. Thus, hepatoprotective activity of CDP-C was studied further. *In vitro* research, CDP-C promoted viability of HepG2 cells. *In vivo* research, CDP-C ameliorated the alterations induced by alcohol, including serological indexes (alanine transaminase, acid phosphatase, γ-glutamyl transpeptidase and triglyceride) and hepatic indicators (superoxide dismutase, malondialdehyde, glutathione S-transferase and triglyceride) in model animals. The prominent microvesicular steatosis and mild necrosis in hepatic histopathology of model animals were also attenuated by CDP-C administration. These findings indicated that CDP-C possessed hepatoprotective activity against chronic hepatic injury induced by alcohol. The underlying mechanism might be that CDP-C can reduce the contents of MDA and TG, and modulate the activities of the relative enzyme. This property might associate with GalUA in CDP-C.

1. Introduction

Cistanche is a perennial holoparasite and mainly distribute in the desert region of northwestern China [1,2]. The stem of *Cistanche* species (*Orobanchaceae*) was first recorded in Shen Nong's Chinese Materia Medica. Cistanche has long been used as a traditional herbal medicine for the treatments of kidney deficiency, impotence, senile constipation, soreness and weakness of waist and knees, and blood deficiency [3,4]. Pharmacological researches revealed that Cistanche has anti-inflammatory activity [5,6], anti-osteoporosis activity [7,8], sedative effect [9], antifatigue activity [10], neuroprotective effect [11]. Besides, polysaccharides from *Cistanche deserticola* (CDPs) has anti-hyperglycemic and hypolipidemic effects [12], immunological activity [13] and proliferation effect on lymphocytes [14].

Alcohol, a worldwide consumed beverage and food additive, induced nearly 2.5 million deaths each year in the world [15,16]. These deaths mainly related to liver disease induced by alcohol abuse [17,18]. Alcohol induced hepatic disease is a complex multistep chronic progression, normally develops from alcoholic steatosis to alcoholic hepatitis and finally to alcoholic cirrhosis [19]. Thus, identifying active natural products for those alcohol consumers to prevent or slow down the progression of alcoholic liver injury in early stage is a beneficial management strategy.

Cistanche deserticola Y. C. Ma and *Cistanche tubulosa* (Schrenk) Wight are two of medicinal species recorded in Chinese Pharmacopoeia [3]. Many papers have elucidated the hepatoprotective activities of *C. tubulosa* [20–24] and *C. deserticola* [25,26]. But these hepatoprotective researches always aimed at acute liver injury induced by carbon tetrachloride (CCl_4) or D-galactosamine and lipopolysaccharide, but not concerned about the chronic liver injury related with alcohol abuse. In addition, these reports were mainly focused on the mechanism of phenylethanoid glycosides (PhGs) rather than the CDPs. Therefore, in the present study, the preliminary characterizations of CDPs and their antioxidant activ-

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ities (*in vitro*) were investigated. Moreover, liver injury model of ICR mice induced by white spirit wine was established to examine the hepatoprotective activity of CDPs against chronic liver injury induced by alcohol.

2. Materials and methods

2.1. Materials

The stems of *C. deserticola* were collected from Alashan League, Inner Mongolia of China, and identified by Prof. Xiaodong Wang (Division of Biorefinery Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing, P. R. China).

Dimethyl sulfoxide (DMSO), 2, 2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) and 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Dulbecco's Modified Eagle Medium (DMEM) and fetal bovine serum (FBS) were purchased from Invitrogen, Inc. Er Guo-tou white spirit was purchased from Beijing Red Star Co., LTD. Bicyclol was purchased from Beijing Union Pharmaceutical Factory. Aqueous solutions were prepared with ultra-pure water from a Milli-Q water purification system (Millipore, Bedford, MA, USA). All other reagents were of analytical grade.

2.2. CDPs extraction and fractional filtration

The crude CDPs were prepared according to the previous reported method of our group [27] with slight modification. Briefly, the powder (40 mesh) of *C. deserticola* (50 kg) was ultrasonically (40 kHz and 6 kW) extracted with 1000 L of aqueous ethanol solution (50%, v/v) at 60 °C for 120 min. Polysaccharides were separated from the extract with macroporous resin (HPD 300). Polysaccharide solution was concentrated under reduced pressure, deproteinized using Sevag's method [28], and freeze dried the product was called crude CDPs.

Then 50 g of the crude CDPs was dissolved in 2.5 L ultrapure water and successively separated with the microfiltration, ultra-filtration and nanofiltration (nominal molecular weight cut-offs were 300 kDa, 10 kDa and 200 Da. Effective membrane area was 0.625 m^2). Retained solution of microfiltration was lyophilized and called CDP-A. Permeated solution of microfiltration was filtrated with ultra-filtration, while retained solution was lyophilized and named as CDP-B. Permeated solution of ultra-filtration was filtrated with nanofiltration, retained solution was lyophilized and named as CDP-C.

2.3. Preliminary characterizations of CDPs

The molecular sizes of CDPs were evaluated according to the previous reported method of our group [29]. Monosaccharide compositions were analyzed using the method described by P. zhang et al. [30]. Purities were determined according to the phenol-sulfuric acid method [31]. The protein contents were determined using the method mentioned by Bradford and Maja Kozarski [32,33]. FT-IR spectra of CDPs were captured with a Fourier Transform-Infrared Spectrometer (FT/IR-660 Plus, JASCO) in the range of 400–4000 cm^{-1}.

2.4. Antioxidant activities

The scavenging effects of the CDPs on hydroxyl, superoxide anion, DPPH and ABTS radical were evaluated according to the methods described by Sun [34],Wang [35], Yap [36] and Fatiha [37], respectively.

2.5. Hepatoprotective activity of CDP-C in vitro

On the basis of the results of antioxidant assay, CDP-C was selected to study the hepatoprotective activity. HepG2 cells (purchased from China Center for Type Culture Collection, Beijing, China) were cultured in DMEM containing heat inactivited FBS (10%), streptomycin (0.1 μg/mL), penicillin (100 IU/mL) and non-essential amino acid. The cells were incubated in humidified atmosphere of 5% CO_2 at 37 °C, harvested at the exponential growth phase, seeded into 96-well plates (4×10^4 cells per well, 100 μL) and incubated for 24 h. Cells of negative group were treated with alcohol (final concentration was 3.5%, v/v) while naive group treated with water. Cells of positive group were treated with alcohol and bicyclol (final concentration was 200 μg/mL). Cell of the four test groups were treated with alcohol and CDP-C (final concentration of CDP-C was 0.11, 0.3333, 1.00 and 3.00 mg/mL). All cells were cultured for another 48 h.

The viability of HepG2 cells were determined by MTT assay method mentioned by Tong et al. [38] with slight modification. Briefly, MTT (5 mg/mL, 20 μL per well) was added into the cell-seeded 96-well plates, and the cells were incubated for 4 h. The solutions were removed and DMSO (150 μL/well) was added. The absorbance of each well was measured at 492 nm using a 96-well plate reader (Thermo Scientific, America). The cell viability was calculated using the following formula:

$$\text{Cellviability}(\%) = (A_{\text{sample}} - A_{\text{blank}}) \times 100/(A_{\text{naive}} - A_{\text{blank}})$$

Where A_{sample} was the absorbance of the experimental group; A_{naive} was the absorbance of the control group without sample; A_{blank} was the absorbance of culture medium without any sample and seeded cell.

2.6. Hepatoprotective activity of CDP-C in vivo

2.6.1. Animals

Adult female ICR mice (22–25 g, purchased from the Beijing Vital River Laboratory Animal Technology Co. Ltd. Animal license number was 11400700128) were utilized. Animals were housed in an environmentally-controlled room with feed and water ad libitum (relative humidity was 40–60%, 22–26 °C), air ventilation was 12–18 times/h, and light irradiation condition was a 12 h light/dark cycle of 150–300 lx. The mice were acclimatized to the animal room conditions for 7 days prior to experiments. All procedures involving animals throughout the experiments were conducted in strict accordance with the rules for Use of Laboratory Animals as adopted and promulgated by the United States National Institutes of Health.

2.6.2. Experimental design

ICR mice were randomly divided into the six groups (naive group, negative control group, positive control group and three test group) with 10 animals in each group. The naive group was orally administered with distilled water. Negative control group was orally administered with alcohol (Er Guo-tou white spirit, 56%, 6 mL/kg). Positive control group was orally administered with bicyclol (300 mg/kg) and 5 h later with alcohol. Three test groups were orally administered with CDP-C at a dose of 200, 600, 1800 mg/kg and 5 h later with alcohol. All mice were administered for 31 consecutive days.

2.6.3. Determination of serological indexes

About 4 h after last alcoholic treatment, blood was collected from eye socket and centrifuged at 3000*g* for 15 min. After the