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The comparison of antioxidative and hepatoprotective activities of *Codonopsis pilosula* polysaccharide (CP) and sulfated CP

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ABSTRACT

Codonopsis pilosula polysaccharide (CP) was extracted, purified and modified by chlorosulfonic acid-pyridine method to obtain a sulfated CP (sCP). Their antioxidative activities in vitro were compared through the free radical-scavenging test. The results demonstrated that the scavenging capabilities of sCP were significantly stronger than those of CP. In vivo test, the mice hepatic injury model was prepared by BCG/LPS method, then administrated respectively with sCP and CP at three dosages, the biochemical indexes in serum, antioxidative indexes in liver homogenate and histopathological change in liver of the mice were compared. The results showed that in high (200 mg/kg) and middle (150 mg/kg) dosages of sCP groups, the contents of ALT, AST and TNF-α in serum and MDA in liver homogenate were significantly lower than those in the model group and numerically lower than those in the CP groups, the activities of SOD and GSH-Px in liver homogenate were significantly higher than those in the model group and numerically higher than those in the CP groups. In the model group there were obvious pathological changes in the liver, while in the sCP groups were near normal. These results indicate that sCP and CP possess antioxidative activity in vitro and in vivo, the activity of sCP is stronger than that of CP and sulfation modification can enhance the antioxidative and hepatoprotective activities of *Codonopsis pilosula* polysaccharide.

1. Introduction

Hepatic injury is a prevalent ailment in the whole world and often caused by viral infection, metabolic disorders, drugs and alcohol abuse and so on, especially in the chronic case which will eventually lead to liver fibrosis and cirrhosis [1,2]. The pathogenesis of hepatic injury is not fully clear, but it is closely linked to the oxidative stress and inflammatory reaction. The excessive oxidative stress caused by reactive oxygen species and reactive nitrogen species increase the risk for hepatic injury through oxidation of biomolecules including DNA, lipids, and proteins [3], proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-1, have been linked to promote hepatic injury [4,5].

As the main active ingredient of Chinese herbal medicine, polysaccharide has anti-tumor, anti-viral, anti-inflammatory, immunomodulatory, anti-oxidation [6–8] and other biological activities. Many studies have shown that the molecule modification can significantly enhance the biological activity of polysaccharide, such as anti-viral [9], immune-enhancing [10] and anti-oxidant effects [11]. Therefore sulfation modification has been widely used [12].

Codonopsis pilosula has been used in traditional Chinese medicine for thousands of years. It contains a variety of active ingredients, such as polysaccharides, phenols, saponins, alkaloids and so on. *C. pilosula* polysaccharide (CP) is an important active ingredient and possesses antioxidant, immune-enhancing, anti-tumor and other pharmacological activities [13,14]. The water-soluble CP was consist of a backbone (1→3)-linked-β-D-galactopyranosyl, (1→2,3)-linked-β-D-galactopyranosyl and (1→3)-linked-α-D-rhamnopyranosyl residues, with a molecular mass of 1.1×10^4 Da [15]. Our previous studies have confirmed that sulfation modification can enhance the activity of CP [16].

Abbreviations: CP, *Codonopsis pilosula* polysaccharide; sCP, sulfated CP; TNF-α, tumor necrosis factor-α; BCG, Bacille–Calmette–Guérin; LPS, lipopolysaccharide; CSA-Pyr, chlorosulfonic acid-pyridine; DPPH, 2,2-Diphenyl-1-picrylhydrazyl; ABTS, 2,2′-Azinobis-(3-ethylbenZthiazoline-6-sulphonate); T-SOD, total superoxide dismutase; MDA, malondialdehyde; GSH-Px, glutathione peroxidase; FT-IR, Fourier transform-infrared spectroscopy; VC, vitamin C; NC, negative control; MC, model control; PC, positive control; TP, total protein; ALP, alkaline phosphatase; ALT, aspartate aminotransferase; AST, alanine aminotransferase; HE, hematoxylin and eosin.

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In the present study, CP was extracted, purified and modified by chlorosulfonic acid-pyridine (CSA-Pyr) method to obtain a sulfated CP (sCP). Their antioxidant activities in vitro were compared through the free radical-scavenging assay, and the hepatoprotective effects in vivo were evaluated using mice hepatic injury model prepared by Bacille–Calmette–Guérin/lipopolysaccharide (BCG/LPS) method. The purpose of this research is to confirm whether sulfation modification can enhance the antioxidative and hepatoprotective activities of CP, and offer theoretical evidences for development of new-type antioxidative drug.

2. Materials and methods

2.1. Materials and reagents

C. pilosula was the product of Zhejiang Qianjiang Chinese Traditional Medicinal Electuary Co., Ltd., standard No. 130826. The reagents of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-Azinobis-(3-ethylbenZthiazoline-6-sulphonate) (ABTS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). ABTS radical solution was prepared by mixing ABTS aqueous solution (final concentration 7.4 mmol·L^{-1}) with potassium persulphate (final concentration 2.6 mmol·L^{-1}), and the mixture was incubated for 24 h in the dark at room temperature before use. BCG vaccine was purchased from Shanghai Institute of Biological Products of China. Bifendate Pills was purchased from Beijing Union Pharmaceutical Factory, standard No. 14010101. Total superoxide dismutase (T-SOD), malondialdehyde (MDA) and glutathione peroxidase (GSH-Px) kits were purchased from Nanjing Jiancheng Bioengineering Institute of China. TNF-α kit was purchased from Suzhou Calvin Biotechnology Co., Ltd. of China.

2.2. Preparation of CP

Crude CP was extracted by water decoction and alcohol precipitation method, then using Sevage's method to remove the protein, active carbon absorption to remove the pigment and through Sephadex G-75 column [15]. The eluate was dialyzed in a dialysis sack against distilled water for 24 h and lyophilized to get the purified CP [17,18]. The polysaccharide content of CP was 87.4% measured by the phenol-sulfuric acid method [19].

2.3. Preparation of sCP

sCP was prepared using CSA-Pyr method [20,21]. In brief, CP was resuspended in N,N-dimethyl-formamide and added into three-necked flask filled with CSA-Pyr of 1:6 (v/v) sulfating reagent in ice bath, the mixture was stirred for 3 h at 80 °C then pH was adjusted to 7–8 with saturated NaOH solution and cooled to room temperature, 3-fold volume of dehydrated alcohol was added, the precipitation was dialyzed in dialysis sack against distilled water for 3 days, and dried in LGJ-25 vacuum freeze-drying machine (Xiamen, China).

The polysaccharide content of sCP was 63.2% as measured by the phenol-sulfuric acid method, and the degree of substitution of sCP was 1.83 measured by barium chloride-gelatin assay [22]. The structure of sCP was identified by Fourier transform-infrared spectroscopy (FT-IR) [23]. In the FT-IR spectra of sCP, a large S═O stretching vibration appeared at 1229.66 cm^{-1} and a symmetrical C–O–S stretching vibration appeared at 811.30 cm^{-1} (Fig. 1). These indicated that sCP was successfully modified in sulfation.

2.4. Determination of antioxidant activity in vitro

2.4.1. DPPH radical scavenging assay

The DPPH radical scavenging activities of sCP and CP were measured according to the method described by Shimada with some modifications [24]. The sCP and CP were dissolved with distilled water into 0.010, 0.020, 0.039, 0.078, 0.156, 0.313, 0.625, 1.25, 2.5 and 5 mg/mL as sample solution, respectively. 2 mL of the sample solution was mixed with 2 mL of 0.1 mmol·L^{-1} DPPH ethanol solution. After incubated for 30 min in the dark at room temperature, the absorbance of the mixture (A_1) was measured at 570 nm using the Biomate 3S spectrophotometer (Thermo scientific, USA). The absorbance of the blank control (A_0, water instead of sample solution) was measured by same method. The scavenging activity of DPPH radical was calculated according to the following equation. Scavenging activity (%) = $(1 - A_1 / A_0) \times 100$. Vitamin C (VC) was used as positive control.

2.4.2. Hydroxyl radical scavenging assay

The hydroxyl radical scavenging activities of sCP and CP were õmeasured according to Fenton's reaction [25] with some modifications. The hydroxyl radical was generated in the mixture of 1 mL of 0.75 mmol·L^{-1} 1,10-phenanthroline, 1.5 mL of 0.15 mol·L^{-1} sodium phosphate buffer (pH 7.4), 1 mL of 0.75 mmol·L^{-1} $FeSO_4$ and 1 mL of H_2O_2 (0.01%, v/v). The samples were dissolved in distilled water as described above. After addition of 1 mL sample above-mentioned, the mixture was incubated at 37 °C for 30 min in the dark. The absorbance of the mixture (A_1) was measured at 510 nm, the absorbance of the blank control (A_0, water instead of sample solution) and H_2O_2 control (A_2, water instead of H_2O_2) were measured by same method. The scavenging activity of hydroxyl radical was calculated according to following formula. Scavenging activity (%) = $(A_1 - A_0) / (A_2 - A_0) \times 100$. VC was used as positive control.

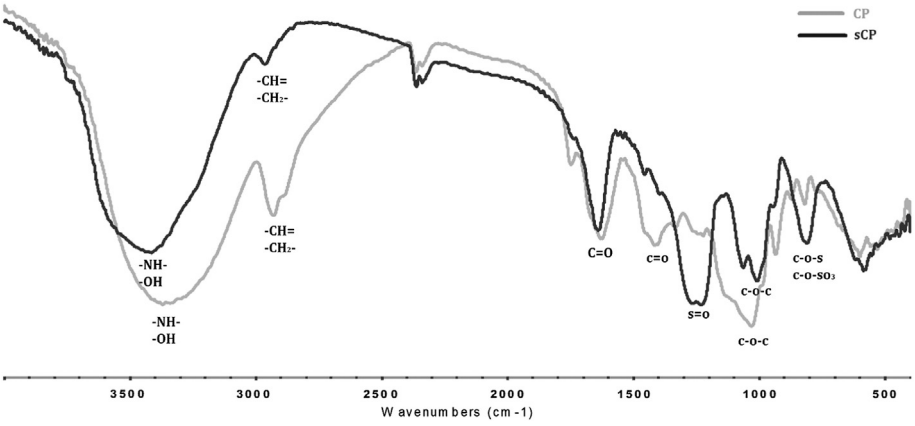

Fig. 1. Fourier transform-infrared spectra of CP and sCP. As compared with the spectrum of CP (gray line), in the spectrum of sCP (black line), at around 3400 cm^{-1} the strong band due to the hydroxyl stretching vibration is decreased; at 1229.66 cm^{-1} there appeared a large S═O stretching vibration and at 811.30 cm^{-1} there appeared a symmetrical C–O–S stretching vibration.