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Anti-diabetic effect of *Coptis Chinensis* polysaccharide in high-fat diet with STZ-induced diabetic mice

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ABSTRACT

For the past few years, numerous polysaccharides and polysaccharide-protein complexes have been isolated from plant or animal and used as a promising source of therapeutic agents for diabetes mellitus (DM). In this study, a water-soluble polysaccharide, named as CCPW-1, was extracted and fractioned from the roots of *Coptis Chinensis* by DEAE Sepharose Fast Flow anion-exchange and Sephadex G-100 column chromatography. The determination of the monosaccharide composition in CCPW-1 with gas chromatography (GC) showed that CCPW-1 was composed of glucose (54.8%), arabinose (22.3%), xylose (11.5%), galactose (7.6%) and galacturonic acid (3.8%). Diabetic mice induced by high-fat diet (HFD) with streptozotocin (STZ) were administered CCPW-1 (100, 50, 25 mg/kg b.w.). Effects of CCPW-1 on bodyweight, fasting blood glucose (FBG), oral glucose tolerance test (OGTT), fasting serum insulin (FINS), total glycerin (TG), total cholesterol (TC), super oxygen dehydrogenises (SOD), catalase (CAT) and methane dicarboxylic aldehyde (MDA) were investigated. CCPW-1 could improve the bodyweight, reduce the content of FBG and enhance FINS level. Meanwhile, CCPW-1 significantly suppressed the rise in blood glucose after 30 min in OGTT. TG and TC levels of diabetic mice also decreased after CCPW-1 treatment. Furthermore, CCPW-1 showed an obvious antioxidant effect through increasing SOD, CAT activities and decreasing MDA content in pancreas. These results indicate that CCPW-1 could be developed to a potent drug used for the treatment of DM in the future.

1. Introduction

Now there has been a sharp increase of diabetes across the world, paralleling the overweight and obesity. It is estimated that the number of diabetics will rise to 380 million worldwide by 2025. DM is a multi-factorial disease characterized by hyperglycemia resulting from defects of insulin secretion and insulin action, which leads to impaired functions in carbohydrate, lipid and protein metabolism [1]. The present therapies for DM include the use of insulin and oral hypoglycemia agents, but these approaches currently used in clinical practice can also induce certain negative effects, such as hypoglycemia, liver problems, lactic acidosis and diarrhea [2,3]. So in order to keep away from these negative effects, people begin to focus more on the traditional herbal medicine. And many herbal medicines have already been investigated and recommended for the treatment of diabetes and shown a good future [4,5]. Therefore, it is vital to seek for natural and non-toxic anti-diabetic medicines for diabetic therapy.

Coptis Chinensis, also known as huanglian, one of the most widely used traditional Chinese medicines, has attracted much attention because of its multiple pharmacological effects, such as anti-diabetic, anti-inflammation, anti-cancer, and so on [6–8]. Previous studies have confirmed that various alkaloids, including berberine, coptisine, palmatine and jatrorrhizine, could have beneficial physiological effects on DM and its complications [9,10]. It is reported that *Coptis Chinensis* decoction can produce much more hypoglycemic activities than berberine, jatrorrhizine and their combinations [11]. It is also demonstrated that its non-alkaloid extracts may significantly promote the glucose consumptions of the cultivated 3T3-L1 adipocytes [12]. The studies described above implied that there would be other constituents in *Coptis Chinensis*, which may contribute somehow to the treatment of DM and its complications. In recent years, the anti-diabetic effect of polysaccharide has widely been reported [13–15]. To the best of our knowledge, a few scholars have reported the extraction of polysaccharide from *Coptis Chinensis* and its anti-diabetic activity in diabetic mice so far. Due to these reasons, we tried to isolate

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a polysaccharide from *Coptis Chinensis* roots, and then, evaluate its hypoglycemic and antioxidant effects on HFD/STZ-induced diabetic mice.

2. Experiments

2.1. Materials and chemicals

Coptis Chinensis was purchased from a local medicine market and identified according to the identification standards of Pharmacopeia of the People's Republic of China. DEAE Sepharose Fast Flow and Sephadex G-100 were purchased from Amersham Pharmacia Co. (Sweden). The standard sugars and STZ were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Reagents for serum insulin were purchased from Invitrogen Biotech Co (Camarillo, CA, USA). TG, TC, SOD, CAT and MDA test kits were obtained from Huili Biotech Co. (Changchun, Jilin, China) and iodine [^{125}I] insulin radioimmunoassay kit was purchased from Jiancheng Biotech Co. (Nanjing, Jiangsu, China). All of other reagents were analytical grade from Sinopharm Chemical Reagent Co. (Beijing, China).

2.2. Extraction, isolation and purification of polysaccharide

The air dried and crushed *Coptis Chinensis* were exhaustively extracted with 90% EtOH. After being filtered, the residues were dried and extracted with distilled water at 90 °C three times and 1 h for each time. After centrifugation (3500 rpm, 10 min, at 20 °C), the supernatant was concentrated, and precipitated with 95% EtOH in 5-fold volume of the supernatant at 4 °C for 24 h. Then, the precipitate collected by centrifugation was deproteinated by the Sevag method [16], followed by exhaustive dialysis with water for 48 h. The precipitate was washed with absolute EtOH, acetone, and ether, and finally a crude polysaccharide was obtained, named as CCPW.

CCPW was dissolved in distilled water, centrifuged, and then, the supernatant was added to a column of DEAE Sepharose Fast Flow equilibrated with 0.02 M PBS (pH 7.2). After being loaded with sample, the column was eluted with 0.02 M PBS, and then followed by the elution with continuous gradient concentrations of NaCl aqueous solution (0.5–1.0 M) stepwise at 2.0 ml/min. The samples eluted by PBS were collected and purified by gel permeation chromatography on a Sephadex G-100 column (2.6 cm × 100 cm) with 0.02 M PBS at a flow rate of 2 ml/min. One main fraction containing large amount of sugar was collected, dialyzed and precipitated with ethanol to obtain a purified polysaccharide, named as CCPW-1. The collection of each tube was monitored by the phenol–sulfuric acid assay at 490 nm.

2.3. General analytical methods

UV–vis absorption spectrum was recorded with a Shimadzu 2550 spectrophotometer. GC was performed on Agilent 6890 N instrument equipped with a HP-5 column (30 m × 0.25 mm × 0.25 μm) and a flame ionization detector (FID). The column temperature was kept at 120 °C for 2 min and rose to 250 °C (maintained for 3 min) gradually at a rate of 8 °C/min. The injector and detector heater temperature was 250 °C and 290 °C, respectively. The rate of N_2 carrier gas was 1.1 ml/min. Inositol was used as the internal standard. The percentage of monosaccharide in the sample was calculated based on the peak areas using response factors.

The content of total carbohydrate, uronic acid, and protein were assayed by the phenol–sulfuric acid [17], carbazole reaction [18], and Bradford methods [19] respectively, using Glu, GalA, and bovine serum albumin (BSA) as the respective standards.

2.4. Monosaccharide composition of CCPW-1

The monosaccharide composition analysis was performed by gas chromatography. CCPW-1 (10 mg) was firstly hydrolyzed into monosaccharide with 2 M TFA (100 °C, 2 h) by using the methods described by Jones and Oades [20,21]. Then the hydrolyzed product was reduced with KBH_4 (30 mg), followed by neutralization with dilute acetic acid and evaporated at 45 °C after adding 1 mg myo-inositol and 0.1 M Na_2CO_3 (1 ml) at 30 °C with stirring for 45 min. The residue was concentrated by adding methanol. Finally the reduced products (alditols) were added with 1:1 pyridine–propylamine at 55 °C with stirring for 30 min, and acetylated with 1:1 pyridine–acetic anhydride in a boiling water bath for 1 h. The acetylated products were analyzed by GC, and identified and estimated with myo-inositol as the internal standard [22].

2.5. Animal model [23,24]

Male ICR mice (18–22 g) were purchased from the Experimental Animal Breeding Centre, Jilin University (Changchun, Jilin, China). All mice were housed in an air-conditioned room at 22 ± 1 °C, with humidity of 50 ± 10%, and a constant 12 h light and 12 h dark cycle. They were fed with diet and given tap water ad libitum. Normal-pellet diet (NPD), consisting of 5% fat, 53% carbohydrate, 23% protein and with total calorific value 25.0 kJ/kg, and HFD, consisting of 22% fat, 48% carbohydrate, and with 20% protein with total calorific value 44.3 kJ/kg were also ordered from the Experimental Animal Breeding Centre, Jilin University.

The mice were randomly divided into 2 groups. One group (n = 6, normal control) was fed with NPD and another group (n = 40) were fed with HFD. After 4 weeks, the mice fed with HFD were injected intraperitoneally with STZ dissolved in citrate buffer (pH 4.5) at the dose of 30 mg/kg b.w., and their FBG levels were measured on week 4 after the injection. The mice fed with NPD were injected intraperitoneally with the citrate buffer vehicle. HFD-fed mice with FBG levels above 7.8 mM were randomly divided into 5 groups (n = 6 each) and were fed with HFD continuously. One group was used as a diabetic control and one group was given glibenclamide 5 mg/kg b.w. Other 3 groups were orally gavaged with CCPW-1 at the doses of 100, 50 and 25 mg/kg b.w., respectively. The mice were grouped as follows:

Group I: Normal control mice treated with vehicle alone (NC).
Group II: Diabetic control mice treated with vehicle alone (DC).
Group III: Diabetic mice were given glibenclamide 5 mg/kg b.w. (DG).
Group IV: Diabetic mice were given CCPW-1 100 mg/kg b.w. (HCCPW-1)
Group V: Diabetic mice were given CCPW-1 50 mg/kg b.w. (MCCPW-1)
Group VI: Diabetic mice were given CCPW-1 25 mg/kg b.w. (LCCPW-1)

CCPW-1 and glibenclamide were given orally once a day for 28 days. On the 28th day of the experiment, the animals were deprived of food overnight before being sacrificed. Blood samples obtained from tails were separated by centrifugation for 5 min and kept at −80 °C. The pancreases were immediately separated, collected and stored in liquid nitrogen for the further analysis.

2.6. Fasting blood glucose and oral glucose tolerance test

Throughout the 4 weeks treatment period, FBG was measured weekly on tail vein blood samples. OGTT was performed in the last week of treatment. After a 12-h fast, the animals were orally gavaged with 2 g/kg b.w. of glucose solution (40%, wt/vol). Blood