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Chemical characterization of *Lycium barbarum* polysaccharides and their reducing myocardial injury in ischemia/reperfusion of rat heart

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ABSTRACT

Polysaccharides were extracted from *Lycium barbarum* fruits in this work. Fourier transform infrared spectroscopy (FT-IR) and high-performance liquid chromatography (HPLC) have been employed to characterize this polysaccharides in the present study. The results of chemical composition indicated that the *L. barbarum* polysaccharides were composed of two kinds of monosaccharides, namely glucose and fructose in molar ratios of 1:2.1. The results indicated that the glucose and fructose were the predominant monosaccharides. IR spectrum of *L. barbarum* polysaccharides revealed a typical peaks of polysaccharides. The results still showed that *L. barbarum* polysaccharides significantly decreased the myocardium LD level, increased Na^+–K^+-ATPase and Ca^{2+}-ATPase activities in heart ischemia reperfusion (IR) rats. In addition, *L. barbarum* polysaccharides still markedly decreased myocardium Bax positive rate and myocardial cell apoptosis and increased Bcl-2 positive rate in a dose-dependent manner. It may be concluded that administration of *L. barbarum* polysaccharides can prevented the development of cardiovascular diseases.

1. Introduction

Heart disease or cardiovascular diseases is the class of diseases that involve the heart or blood vessels (arteries and veins) [1]. However, the rapidly increasing incidence of metabolic disorders such as diabetes, obesity and metabolic syndrome combined with more aggressive treatment of hypertension is shifting the underlying aetiology towards hyperglycaemia and dyslipidaemia. The borderline hypertensive rat (BHR) is used as a model to examine how the interaction of environmental stressors and genetic factors may affect acute and chronic regulation of cardiovascular function [2].

Fruit from *Lycium barbarum* L. in the family Solanaceae is well-known in traditional Chinese herbal medicine and nowadays has been widely used as a popular functional food, with a large variety of beneficial effects, such as reducing blood glucose and serum lipids, anti-aging, immuno-modulating, anticancer, anti-fatigue, and male fertility-facilitating [3–7]. The bioactive components of *L. barbarum* fruit have been mainly attributed to its polysaccharide–protein complex (LBP), which contains several fractions separated by ion exchange chromatography and size exclusion chromatography [8–12]. LBP fractions generally consist of six monosaccharides (galactose, glucose, rhamnose, arabinose, mannose, and xylose) and 18 amino acids [8–10]. Previous studies have shown that LBP can enhance the immune function [9,10], protect liver damage [13], lower blood glucose level [14], reduce the side effects of chemotherapy and radiotherapy [15], and act against cancer [9,10].

In this study, we extracted polysaccharides from *L. barbarum*. Then, we investigated the LD, NO levels and Na^+–K^+-ATPase, Ca^{2+}-ATPase activities in the heart from ischemia–reperfusion injury.

2. Materials and methods

2.1. Material

L. barbarum was purchased from an herbs market in Xi'an city, China.

2.2. Polysaccharide extraction

The dry *L. barbarum* fruits (100 g) were firstly extracted with 300 ml of $CHCl_3$–MeOH (3:1 (v/v)) at 70 °C under reflux for 2 h. The residue was then submitted to an acetone extraction under the same conditions. The lipid-free material was extracted with 400 ml of EtOH (70 °C) and then 500 ml of water (100 °C) for 3 h both under reflux.

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2.3. Analysis of monosaccharide composition

Monosaccharide compositional analyses were conducted via acid digestion on untreated, water-washed, and pretreated and water-washed corn stover. The resulting hydrolyzates were analyzed by HPLC. These procedures were conducted according to the methods of Kaar et al. [16].

2.4. Infrared spectrum analysis of L. barbarum polysaccharides

The infrared spectrum of *L. barbarum* polysaccharides was determined using a Fourier transform infrared spectrophotometer (Bruker, Ettlingen, Germany) equipped with OPUS 3.1 software. Five milligrams of *L. barbarum* polysaccharides were ground with KBr powder and then pressed into pellets for transformation infrared spectrum measurement in a frequency range of 400–4000 cm^{-1} [17].

2.5. Experimental groups and operative procedures

Thirty-two (32) Wistar adult male rats weighing 250 ± 20 g were obtained from the Experimental Research Section of our University, China. Animals were maintained in their cages at a constant room temperature using a 12 h: 12 h light/dark cycle and provided with commercially available rat chow and top water ad libitum. All experimental procedures described below were approved by the Ethical Committee of School of Medicine at the University.

Rats were randomly allocated into the following groups. Control group and three surgical groups. Rat hearts were perfused as previously described. Briefly, the heart was rapidly excised from anesthetized rats, and placed in cold (4 °C) Krebs–Henseleit solution (in mM): NaCl 118, KCl 4.8, KH_2PO_4 1.2, $CaCl_2$ 1.6, $MgSO_4$ 1.2, $NaHCO_3$ 25 and glucose 11.5 mM. Within 2 min after thoracotomy the heart was removed, mounted on the experimental setup and perfused retrogradely at 15 ml/min (Minipuls-3 peristaltic pump; Gilson, Villiers-Le Bel, France) through the aorta with Krebs–Henseleit solution, which was kept at 37 °C and aerated with 95% O_2 + 5% CO_2 to maintain normal pH, pO_2 and pCO_2 levels. Coronary perfusion pressure and left ventricular pressure were measured with two pressure transducers (model HP-1280C; Hewlett-Packard, Waltham, MA, USA) connected to a Hewlett-Packard dynograph. Left ventricular pressure was recorded using a polyethylene catheter with a small latex balloon at the tip (size 3; 2Biological Instr., Besozzo, Varese, Italy) inserted in the left ventricular cavity through the mitral valve opening. The volume of the balloon was adjusted to give peak left ventricular systolic pressure of 85–90 mm Hg with left ventricular end-diastolic pressure of 5–6 mm Hg. Hearts that could not achieve this level of contractile performance (8–10%) were excluded. Left ventricular developed pressure (peak left ventricular systolic pressure minus ventricular end-diastolic pressure) was also calculated.

Surgical rats were further randomly divided into three experimental groups (eight rats per group) as follows: model control, low-dose of polysaccharides treatment group (150 mg/kg bw) and high-dose of polysaccharides treatment group (300 mg/kg bw). At the end of the experiment, the hearts were removed immediately and stored at −70 °C for biochemical measurements.

2.6. Biochemical analysis

Concentrations of nitric oxide (NO–) and lactic acid were measured in perfused culture media by using a fluorometric nitric oxide assay kit.

Na^+/K^+-ATPase activity was assayed according to the literature [18] with minor modifications. Briefly, the assay mixture in a final volume of 1 ml contained microsome (50/∼g) and a buffer of 50 mM Tris/HCl pH 7.4, 20 mM KCl, 100 mM NaCl, 1 mM EGTA, 5 mM $MgCl_2$ and 5 mM ATP. The control tubes contained the same reaction mixture and 1 mM ouabain. The reaction was started by the addition of ATP and the tubes were incubated at 37 °C for l0 min. The reaction was stopped by the addition of 500/∼1 of 20% TCA, and the amount of inorganic phosphate was determined. The Na^+/K^+-ATPase activity (100%) represents the difference between the activity in the presence and absence of 1 mM ouabain. Inhibition percentages were calculated by comparing the activities in the presence of different concentration of ouabain to the 100% value. The experimental data were fitted using ENZFITTER (Biosoft, Elsevier, Cambridge, UK).

Ca^{2+}-ATPase activity was assayed by measuring the release of inorganic phosphate from ATP as reported elsewhere [19]. The incubation mixture contained 40 mM Tris–HCl buffer (pH 7.0), 5 mM $MgCl_2$, 50 mM KCl, 0.1 mM ouabain, 2 mM ATP (neutralized with hydroxide potassium (KOH)), microsomal protein (0.5–0.8 mg/ml) with either 2 mM EGTA or 20 mM $CaCl_2$. The enzyme reaction was started by the addition of microsomes, then carried out for 10 min at 37 °C, and stopped by the addition of ice-cold trichloroacetic acid (TCA, 10%). Inorganic phosphate released by the enzyme reaction was determined. Ca^{2+}-ATPase activity was calculated by subtracting the values obtained with EGTA addition from those obtained with Ca^{2+} addition. Results were expressed as nmoles of inorganic phosphate liberated per min per mg of protein.

Immunohistochemistry was performed on the brain sections adjacent to those used for TUNEL detection and carried out following the avidin–biotin–peroxidase method (Vector Laboratory) [20]. All sections were treated simultaneously. After deparaffinization and dehydration, sections were treated with 0.3% H_2O_2 solution and 1.5% normal goat serum to quench endogenous peroxidase activity and block nonspecific binding and then with each primary antibody in a humidified chamber for 24 h at 4 °C. Rat anti-Bcl-2 and anti-Bax were respectively diluted 1:200 and 1:300. After washing with PBS, the sections were further incubated respectively with biotinylated secondary antibody (goat-anti-rat IgG, 1:200 dilution) and an avidin–biotin–peroxidase complex (ABC kit, Vector, Burlingame, USA) for 30 min and then rinsed in PBS and subsequently exposed to diaminobenzidine and H_2O_2 in 50 mM Tris–HCl (pH 7.6) for 3 min. After the final wash, the immunostained sections were dehydrated in graded ethanol and coverslipped with Permount. Quantitative analysis of Bcl-2- and Bax-positive cells was also performed with the image analyzer system. The average value from adjacent two sections was used for each animal and expressed as the number of cells per 1 mm of CA1 pyramidal cell layer. Negative control sections were incubated without the primary antibody.

Apoptosis rate was performed using flow-cytometric method.

2.7. Data analysis

Results have been expressed as mean ± S.E.M. Statistical analysis was performed by using one-way ANOVA, Duncan's multiple range test and Mann–Whitney *U*-test. A P value 0.05 or less was considered significant.

3. Results and discussion

3.1. Chemical analysis of L. barbarum polysaccharides

HPLC analysis showed *L. barbarum* polysaccharides was composed of two kinds of monosaccharides, namely glucose and fructose in molar ratios of 1:2.1. The results indicated that the glu-