International Journal of Biological Macromolecules 46 (2010) 540–543

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Chemical characterization of lycium barbarum polysaccharides and its inhibition against liver oxidative injury of high-fat mice

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ARTICLE INFO

Article history:
Received 10 January 2010
Received in revised form 12 February 2010
Accepted 22 February 2010
Available online 1 March 2010

Keywords:
High-fat diet
GC–MS
FT-IR
Mice
Lycium barbarum polysaccharides

ABSTRACT

In this study, we investigated chemical structure of lycium barbarum polysaccharides and its modulatory effect on oxidative stress in high-fat mice. The polysaccharides mainly contained xylose and glucose. Little amount of rhamnose, mannose and galactose was observed. The lycium barbarum polysaccharides had IR bands at 800–1200 cm^{-1}, 1450–1800 cm^{-1}, 2500–3000 cm^{-1}, and 3200–3600 cm^{-1}, which were distinctive absorptions of polysaccharides. Rats are fed with high-fat diet for 2 months. Results showed that blood and liver antioxidant enzymes activities and GSH level in model mice significantly decreased, and MDA level significantly increased ($P < 0.01$) compared to normal control mice. Administration of lycium barbarum polysaccharides significantly increased antioxidant enzymes activities and decreased MDA level in mice ($P < 0.01$) compared to model group.

1. Introduction

Formation of reactive oxygen species (ROS) has been proposed to be an important step leading to a variety of degenerative disorders [1,2]. Free radicals are highly reactive, toxic forms of oxygen molecules that bind to and destroy cellular components. Free radicals damage our cells and our DNA, leading to degenerative conditions of the circulatory system, bone and joints, brain and nervous system, eyes and hearing, and our immune system [3]. Supplementing our diets with antioxidants can be very helpful in fighting the damaging effects of free radicals.

High-fat diets have been linked to changes in the fatty acid composition and therewith the fluidity of plasma and intracellular membranes [4], which in turn affect their passive permeability to protons [5] as well as the distribution and dimerization of receptors, activities of mitochondrial membrane-bound enzymes [6–8] and the content of coenzyme Q [9,10].

In recent years, lycium barbarum polysaccharides and its derivatives from animal, plants and fungus have been widely investigated in structures and bioactivities [11,12]. Many beneficial health properties of lycium barbarum polysaccharides are attributed to their antioxidant activities [13–15]. Therefore, in order to evaluate the ROS scavenging activity of lycium barbarum polysaccharides as well as possible oxidative injury protection from ROS, we firstly prepared lycium barbarum polysaccharides, then use the compounds to examine the *in vivo* antioxidant effects on blood and liver in experimental rats fed with high-fat diet.

2. Materials and methods

2.1. Extraction procedure

The lycium barbarum sample is dried. Dried material is dewaxed by refluxing with toluene–EtOH (2:1, v/v) or defatted with chloroform–methanol (2:1, v/v) for 6 h in a Soxhlet apparatus. The resulting material was extracted with boiling water and filtered. Water-soluble polysaccharides are isolated by precipitation of the concentrated supernatant with 4 volumes of 95% ethanol and recovered by centrifugation.

The polysaccharide content of the extract was measured by the sulfuric acid/phenol method. Briefly, polysaccharides were hydrolyzed to sugar aldehyde in the presence of sulfuric acid and condensed with phenol to give a colored complex, which can be quantified by spectrometry at 480 nm. Polysaccharides content was reproducibly found to be of 75–90% (w/w).

Abbreviations: GSH, Reduced glutathione; MDA, Malondialchehyche; GC–MS, Gas chromatographic–mass spectrometric; FT-IR, Fourier Transform Infrared; SOD, Superoxide dismutase; CAT, Catalase; GSH-Px, Glutathione peroxidase; TAOC, Total antioxidant capacity; NO, Nitric oxide.

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doi:10.1016/j.ijbiomac.2010.02.010

2.2. Methylation analysis and IR analysis

Per-O-methylation of the isolated polysaccharides was carried out using powdered NaOH in DMSO-MeI. The products were converted into partially O-methylated aldose acetates by successive treatments with refluxing 2% MeOH–HCl for 2 h and total hydrolysis with 0.5 M H_2SO_4 for 14 h at 100 °C, neutralized ($BaCO_3$), filtered, and the filtrate evaporated to dryness. The products were converted into partially O-methylated alditol acetates as described above, and analyzed by GC–MS.

The polysaccharides samples were measured by Fourier Transform-Infrared Spectroscopy (FT-IR) as a film between two KBr plates on a FT-IR spectrometer (Analect Instruments fx-6 160). The recording was done from 4000 cm^{-1} to 400 cm^{-1} wave number.

2.3. Animals experiment

Fifty kunming mice (3-month-old), weighing 30–37 g, were used after a one week adaptation period (20–23 °C; 12 h light cycle from 09:00 to 21:00). All experiments and the method used in the study were according to the guidelines of the Committee on Care and Use of Laboratory Animals of China.

50 animals were randomized into five groups (each group contained 10 mice): control group, model group, and lycium barbarum-treated groups (low, middle and high doses). The mice of model group were fed with high-fat diet. At the same time, mice in polysaccharides-treated groups (low, middle and high doses) were orally fed with lycium barbarum polysaccharides (50 mg/kg, 100 mg/kg, and 150 mg/kg B.W.) once every day. Mice in normal group were fed with the standard chow. The experiment lasted for 2 months.

At the end of the experiment, blood was taken from the venous sinus of the eyes of each mouse under light ether anesthesia after 10–12 h of fasting. Collected blood was centrifuged for 15 min at 3500 revolutions per minute at 4 °C. The serum sample obtained was used for NO^-, MDA, GSH levels and antioxidant enzymes activities estimation. Livers were removed immediately after euthanasia by cervical dislocation, and washed three times with cold physiological saline (0.9% NaCl). The tissue was then homogenized with a glass-teflon homogenizer at 5000 rpm for 2 min after adding 10 volume of cold 0.9% NaCl. Tissue homogenates were used for NO^-, MDA, GSH levels and antioxidant enzymes activities analysis.

2.4. Biochemical analysis

Accumulated nitrite (NO^-) in tissue was spectrophotometrically determined based on the Griess reaction [16]. The samples (100 μl) were incubated with 100 μl Griess reagent (6 mg/ml) at room temperature for 10 min, and then NO^- concentration was determined by the absorbance at 540 nm. The standard curve was constructed using the known concentrations of sodium nitrite.

GSH was determined by its reaction with 5,5′-dithiobis (2-nitrobenzoic acid) (Ellman's reagent) to yield a yellow chromophore which was measured spectrophotometrically [17]. The serum or supernatant was used for GSH estimation. To 0.1 ml of processed tissue sample, 2 ml of phosphate buffer (pH 8.4), 0.5 ml of 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) and 0.4 ml of double-distilled water were added and the mixture was shaken vigorously on vortex. The absorbance was read at 412 nm within 15 min.

The assays of CAT, SOD, GSH-Px, TAOC activities and MDA level in serum and liver tissues were performed by strictly following the commercial kits instructions.

Table 1
Chemical components of lycium barbarum polysaccharides.

Rhamnose	Xylose	Mannose	Galactose	Glucose
7.5%	31.3%	6.4%	9.3%	49.8%

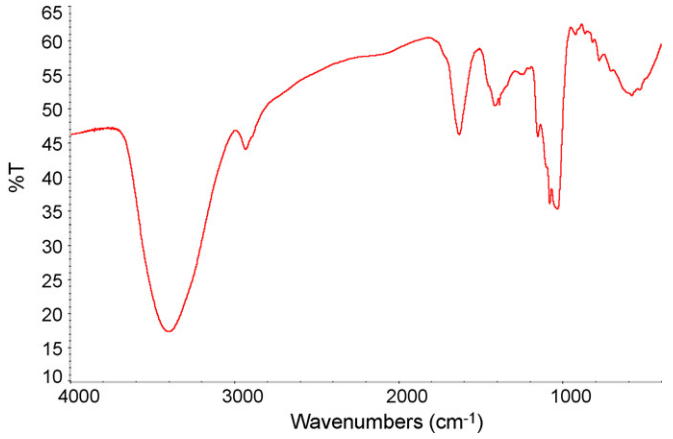

Fig. 1. FT-IR spectra of lycium barbarum polysaccharides.

2.5. Statistical analysis

Data were analyzed using one-way ANOVA and expressed as mean ± SD. Significant difference between groups was detected by Duncan's multiple range test using SPSS 12.0 software. Student's *t* test was used for comparison between two groups. The Mann–Whitney rank sum test was used for the degree of liver oxidative injury. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Chemical components and structure analysis

Table 1 shows the molar percentage of sugars of lycium barbarum polysaccharides after acid hydrolysis. The polysaccharides are rich in xylose and glucose. It was determined more than 10% (molar basis) of xylose and glucose in lycium barbarum polysaccharides but little amount of rhamnose, mannose and galactose was observed.

The lycium barbarum polysaccharides had IR bands at 800–1200 cm^{-1}, 1450–1800 cm^{-1}, 2900–3000 cm^{-1}, and 3200–3600 cm^{-1}, which were distinctive absorptions of polysaccharides. The absorption band at 900 cm^{-1} confirmed the existence of β-glycosidic bond (Fig. 1).

3.2. Antioxidant activities of lycium barbarum polysaccharides in mice

Table 2 showed that antioxidant enzymes activities and GSH level in model mice (group II) significantly ($P < 0.01$) decreased whereas MDA level ($P < 0.01$) increased compared with the control group (I). Administration of lycium barbarum polysaccharides dose-dependently significantly ($P < 0.01$) increased antioxidant enzymes activities and GSH level and reduced MDA level ($P < 0.01$) in mice (groups III–V) compared with the model mice (II).

Table 3 showed that NO^- level in liver and blood of model mice (group II) significantly ($P < 0.01$) increased compared with the control group (I). Administration of lycium barbarum polysaccharides dose-dependently significantly ($P < 0.01$) decreased NO^- level in liver and blood of mice (groups III–V) compared with the model mice (group II).