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Antioxidative and hepatoprotective effects of the polysaccharides from *Zizyphus jujube cv. Shaanbeitanzao*

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ABSTRACT

This study was designed to characterize the chemical composition, antioxidant activity and hepatoprotective effect of the polysaccharides from *Zizyphus jujube cv. Shaanbeitanzao* (ZSP). HPLC analysis showed that ZSP was the heteropolysaccharides with L-arabinose being the main component monosaccharide (50.2%, molar percentage). ZSP displayed strong antioxidant activity in vitro, and the effect was further verified by suppressing CCl_4-induced oxidative stress in liver at three tested doses of ZSP (100, 200, and 400 mg/kg BW) in mice. Administration of ZSP (400 mg/kg) significantly ($p < 0.01$) reduced the activities of CCl_4-elevated alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactic dehydrogenase (LDH) in serum, and hepatic malondialdehyde (MDA) level. Mice treated with ZSP showed a better profile of hepatosomatic index (HI) and antioxidant system with normal glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) activities in liver. These results suggest that ZSP exerts an effective protection against CCl_4-induced hepatic injury by mediating antioxidative and free radical scavenging activities.

1. Introduction

Liver disease is a serious health problem because the liver is an important organ for the biotransformation and detoxification of endogenous and exogenous harmful substances. More and more attention has been paid to liver disease just because of speeding up of the modern pace of life and changing cookbook in recent years (Cemek et al., 2010). It is well-known that free radicals cause cell damage through the mechanisms involving in lipid peroxidation with subsequent tissue injury, especially liver injury (Hsiao et al., 2003). Some antiviral drugs, which have been used to treat liver disease, were shown to have potential adverse effects, especially when administrated for long-term (Muriel & Rivera-Espinoza, 2008). For this reason, natural antioxidants have been a substantial increase as more effective and safe dietary ingredients for alternative therapies of liver disease (Yang, Li, Wang, & Wu, 2010). Therefore, it is of very important and high significant to find out new sources of safe and inexpensive antioxidants of natural origin, such as anthocyanins in color-fleshed potatoes (Lachman et al., 2009), tocotrienols and tocopherols in einkorn and spring wheat varieties (Hejtmánková, Lachman, Hejtmánková, Pivec, & Janovská, 2010) or carotenoids in tomato varieties (Kotíková, Lachman, Hejtmánková, & Hejtmánková, 2011).

Zizyphus jujube, described as the "fruit of life", is a key member of the Chinese herbs which are famous for their hepatoprotective effect. *Z. jujube* belongs to the Rhamnaceae family, and is widely distributed in the temperate and subtropical areas of the North Hemisphere, especially the inland region of North China (Zhang, Jiang, Ye, Ye, & Ren, 2010). *Shaanbeitanzao*, commonly known as a cultivar of *Z. jujube*, is grown in the northwestern part of China along with the Yellow River universally. Besides its edibleness and culinary uses, *Shaanbeitanzao* has also traditionally been used for medicinal purposes for more than 2000 years, where the fruit was made into paste, puree, syrup, and confection, and was consumed for digestion improvement and body maintenance (Huang, Yen, Sheu, & Chau, 2008). However, the active ingredients of *Shaanbeitanzao* responsible for hepatoprotective benefits are not fully clear.

In recent years, natural polysaccharides, which were found largely in fruits and vegetables, have been confirmed to play an important part as free radical scavengers in the prevention of oxidative damage in living organism and can be exploited as novel potential antioxidants, and the effects have something to do with their chemical properties and architectural characteristics (Li, Liu, Fan, Ai, & Shan, 2011). Therefore, discovery and assessment of natural polysaccharides as new safe compounds for functional foods or medicines have became a hot research field. In this regard, the anticancer and immunological effects of jujube polysaccharides

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have been investigated (Hung, Hsu, Chang, & Chen, 2012; Zhao et al., 2006). It is also of interest that the ethanol extract from jujube fruit showed protective effect against CCl_4-induced hepatic injury in mice by antioxidant mechanism (Shen et al., 2009). More recently, the water extract of jujube was also reported to ameliorate the liver injury induced by ischemia/reperfusion (Chen et al., 2010). Although *Shaanbeitanzao* is largely planted in China, up to now, there is no report available on the chemical composition, antioxidant and hepatoprotective effects of the polysaccharides isolated from it. Therefore, the aim of the present study was firstly to assess the chemical composition properties of ZSP, and its antioxidant activity in vitro. Furthermore, the protective effect of ZSP against CCl_4-induced hepatic damage in mice was also explored for seeking a new hepatoprotective function factor used in food and pharmaceutical industry.

2. Materials and methods

2.1. Materials and chemicals

Z. jujube cv. Shaanbeitanzao in packets (Barcode: 69-247450-44010) was purchased from Chang-An shop of Vanguard, which was harvested from the countryside region of Qingjian County (Northwest of China). D-mannose, D-ribose, L-rhamnose, D-glucuronic acid, D-galacturonic acid, D-glucose, D-xylose, D-galactose, L-arabinose, D-fucose, butylated hydroxytoluene, and ascorbic acid (Vit. C) were purchased from Sigma (St. Louis, USA). Potassium ferricyanide [$K_3Fe(CN)_6$] and trichloroacetic acid (TCA) were obtained from Sigma (Sigma Aldrich GmbH, Sternheim, Germany). 1,1-Diphenyl-2-picryl-hydrazyl (DPPH), nitroblue tetrazolium (NBT), nicotinamide adenine dinucleotide (NADH), and phenazine methosulfate (PMS) were obtained from Applichem (Darmstadt, Germany). Biphenyldicarboxylate Pills (BP) and carbon tetrachloride (CCl_4) were obtained from Zhengjiang Wanbang Pharmaceutical Co. (Wenling, China) and Tianjin Tianli Chemical Reagent Co. (Tianjin, China), respectively. Test kits of alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactic dehydrogenase (LDH), Malondialdehyde (MDA), glutathione peroxidase (GSH-P*x*) and superoxide dismutase (SOD) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Trifluoroacetic acid (TFA), triethylamine (TEA), and 1-phenyl-3-methyl-5-pyrazolone (PMP) were purchased from Merck (Darmstadt, Germany). HPLC grade acetonitrile and methanol were purchased from Honeywell (USA). Other chemicals used in the study were of analytic grade and commercially available.

2.2. Animals

Sixty kunming male mice (weight 18–22 g) were obtained from Experimental Animal Center of Fourth Military Medical University. They were allowed free access to tap water and rodent chow (40% corn flour, 26% wheat flour, 10% bran, 10% fish meal, 10% bean cake, 2% mineral, 1% coarse, and 1% vitamin complex, Qianmin Feed Factory). All the animals were housed under the standard conditions with 12/12 h light-dark cycle at a temperature of 22 ± 2 °C and a humidity of 60 ± 5%. All the experiments were performed in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of People's Republic of China and approved by the Fourth Military Medical University Committee on Animal Care and Use.

2.3. Isolation of the polysaccharides from Z. jujube cv. Shaanbeitanzao

100 g pulp stripped from *Z. jujube cv. Shaanbeitanzao* was dried for 10 min in a domestic microwave oven at 900 W output power, and crushed into powder and soaked in water (1:20, w/v) at 80 °C for 160 min. After three cycles, the incorporate extraction solution was filtrated and concentrated to 10% of the original volume with a rotary evaporator under reduced pressure, and then it was precipitated for three cycles by adding three times of volume of 95% (v/v) ethanol at 4 °C for 24 h. The refined pellets were completely dissolved in appropriate volume of water and intensively dialyzed for 4 days against water (cut-off M_w 8000 Da). The retentate portion was deproteinized by the freeze–thaw process for repeating 10 times followed by filtration. Finally, the extracts were centrifuged at 3000 r/min for 10 min to remove insoluble material and the supernatant was lyophilized in the freeze-dry apparatus to give the refined polysaccharide ZSP.

2.4. Analysis of chemical characterization of ZSP

Total carbohydrate content in ZSP was determined by phenol–sulfuric acid colorimetric method with glucose as a standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). According to the ordinary procedure, absorbance at 490 nm of five calibration solutions of glucose (5–60 μg/mL) was determined, and the standard curve was drawn with absorbance as ordinate and concentration (μg/mL) as abscissa, and the regression equation was obtained. The amount of carbohydrates present in ZSP is determined by comparison with a calibration curve using a spectrophotometer. In brief, 100 mg ZSP was put into volumetric flask (25 mL) to get stock solution once it was completely dissolved in ultrapure water, and the working solution was prepared by diluting the stock solution to the appropriate concentrations. 50 μL of working solution was spiked with 500 μL of 4% phenol, followed by 2.5 mL sulfuric acid. Under the catalysis of sulfuric acid, ZSP was converted to monosaccharides, then to derivants of pyromucic aldehyde immediately after dehydration. The derivants reacted with phenol can produce orange-yellow chemical compounds, and the carbohydrate content of ZSP can be obtained after the determination of absorbance of the orange-yellow chemical compounds.

Monosaccharide composition of ZSP was analyzed by HPLC as our previous procedures (Lv et al., 2009). ZSP sample (20 mg) together with 1.0 mg fucose as internal standard was hydrolyzed with 2 mL of 3 M TFA at 95 °C for 8 h in an ampoule (5 mL) sealed under a nitrogen atmosphere. The released monosaccharides were derivatized with PMP to gain strong UV (Lv et al., 2009). Briefly, the hydrolyzed ZSP solution (100 μL) was spiked with 0.3 M aqueous NaOH (300 μL), and 0.5 M methanol solution of PMP (200 μL). Each mixture was allowed to react for 60 min at 70 °C, and subsequently neutralized with 300 μL of 0.3 M HCl. The resulting solution was extracted with 1 mL chloroform, and the aqueous layer was filtered through a 0.45 μm membrane for HPLC analysis. The analysis of PMP-labeled monosaccharides was performed on a Shimadzu LC-2010A HPLC system. The analytical column used was a RP-C_{18} column (4.6 mm i.d. × 250 mm, 5 μm, Venusil, USA), and the separation was performed at 35 °C. The mobile phase A consisted of acetonitrile, and mobile phase B was 3.3 mM KH_2PO_4–3.9 mM triethylamine buffer containing 10% acetonitrile using a gradient elution of 93-93-91-91% B by a linear decrease from 0-7-9-30 min. Elution was carried out at a flow rate of 1.0 mL/min, and the wavelength for UV detection was 250 nm. The injection volume was 15 μL.

2.5. Evaluation of in vitro antioxidant activity of ZSP

2.5.1. Scavenging activity on DPPH radical

The assay was performed as described by Shimada, Fujikawa, Yahara, and Nakamura (1992). Briefly, 1.0 mL of ZSP solution at various concentrations (0–200 μg/mL) was mixed with 3.0 mL of 0.1 mM DPPH in aqueous methanol. Absorbance at 517 nm was