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Hepatoprotective effects of polysaccharides extracted from *Zizyphus jujube cv. Huanghetanzao*

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ABSTRACT

Jujube polysaccharides have been proved to have various bioactivities. This study was designed to evaluate the chemical composition and hepatoprotective function of the polysaccharides extracted from *Zizyphus jujube cv. Huanghetanzao* (HJP). The composition of HJP was determined as heteropolysaccharides with galactose and arabinose being the main components. The pretreatment of mice with HJP significantly ($p < 0.01$) reduced the activities of serum hepatic AST, ALT, and LDH induced by CCl_4 or acetaminophen (APAP) while the commercial liver-injury treatment drug silybin did not show any prevention effects. Mechanistic study results indicate that the administration of the CCl_4- or APAP-injured mice with HJP enhanced SOD and GSH-Px and decreased MDA, indicating that anti-oxidation and detoxification could be the pathways for the liver protection observed. In addition, the liver prevention and treatment effects of HJP on the liver damage induced by CCl_4 or APAP obtained from the liver enzyme analyses were confirmed by the hepatic histopathology studies in mice. Therefore, HJP could be used as a prevention and treatment agent for liver injury induced by liver toxic chemicals and drugs.

1. Introduction

Liver injuries have become one of the most serious health problems as the organ has various remarkable functions in biotransformation (amino acid metabolism, lipid metabolism, and glycolysis) and detoxification of harmful substances (drug metabolism). Recently, more attention has been paid to acute and chronic liver injuries [1] derived from the exposure of environmental toxic chemicals and toxins as well as drug-induced liver side effects. The medical treatments for the injuries are always difficult to deal with and have limited efficacy. Therefore, there is considerable interest in complementary and alternative medicines for the treatment of liver injuries. In the search of remediation agents for liver injuries, effective and safe dietary ingredients have been evaluated for therapies of liver injuries as the "natural liver-protection drugs". Some successful liver protection agents isolated from herbal plants include curcumin [2], glycyrrhizin [3], resveratrol [4], silybin [5] and so on. Tetrachloromethane (CCl_4) and acetaminophen (APAP) have commonly chosen as the hepatotoxic model compounds of chemicals and drugs, respectively. Extensive literature indicates that as a metabolic product of CCl_4, trichloromethyl radical and trichloromethyl peroxy radical are formed by the cytochrome P450 in the liver during the lipid peroxidation process [6]. The trichloromethyl free radical is believed to cause liver cell necrosis [7]. APAP is the most widely used drug in the world for reducing pain and fever. APAP is mainly metabolized *in vivo* by glucuronidation and sulfation pathways. APAP is partly metabolized by cytochrome P450 (CYP 2E1) to produce toxic metabolites such as N-acetyl-p-benzoquinone imine (NAPQI) [8,9]. The high reactivity of NAPQI causes the GSH depletion in liver covalent binding to intracellular proteins, lipids, and nucleic acids. Several proteins have been confirmed as targets of NAPQI by the immunological and proteomic techniques [10,11]. Harmful biotransformations of APAP *in vivo* disrupt the basic function of hepatocytes and subsequently cause liver injury and hepatic necrosis [12,13]. In terms of blood chemistry indicators of liver injuries, the intake of the large-dose APAP significantly increases transaminase (AST/ALT) and lactate dehydrogenase (LDH) in serum [14], and decreases glutathione (GSH) in liver [15].

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Zizyphus jujube belongs to plants of Rhamnaceae and has been grown in China for over 4000 years [16]. It consists of polysaccharides [17], amino acids [18], triterpenic acid [19], phenolic acids [20], flavonoids [21], mineral constituents [22], crude fiber and essential vitamins. Different bioactive ingredients have been determined in the fruits, seeds and leaves and therefore extracts from all three parts of *Z. jujube* plants could be used as medicinal foods or health supplements either alone or in combination with other herbs [23]. *Z. jujube* fruits can be consumed as food or medicinal food in some regions around world. The daily human consumption amount of fruits or related bioactive ingredients can only be defined through the future systematic clinical research. Jujube has been reported to have a variety of pharmacological properties such as anti-inflammatory [23], anti-oxidative [24], hepatoprotective [25], gastrointestinal protective [26], and immunological activities [27]. In recent years, polysaccharides have been found to play an important role as free radical scavengers in the prevention and treatment of oxidative damage in living organism and can be developed as novel antioxidants [28]. As one of the species of *Z. jujube*, *Huanghetanzao* is widely planted in the Yellow River basin. However, the main ingredients and *in vivo* hepatoprotective effects of *Huanghetanzao* are still not very clear.

In this study, the physicochemical properties of the polysaccharides extracted from *Z. jujube cv. Huanghetanzao* (HJP) were investigated. Besides, the protective effects against the CCl_4 and APAP-induced hepatic injuries were studied in mice. This work was expected to generate initial scientific information for further development of the polysaccaharides (HJP) isolated from *Z. jujube cv. Huanghetanzao* as a potential hepatoprotective agent.

2. Materials and methods

2.1. Materials and chemicals

Z. jujube cv. Huanghetanzao was obtained from Jiaxian, Shanxi, China. D-mannose, D-rhamnose, D-galactose, D-galacturonic acid, D-glucose, L-arabinose, and 1-phenyl-3-methyl-5-pyrazolone (PMP) were bought from Sigma–Aldrich (St. Louis, USA). HPLC grade acetonitrile and CCl_4 were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Hydroxypropyl methylcellulose (HPMC, hypromellose 4000 mPa's), polyvinylpyrrolidone (PVP K30), and APAP were produced by Aladdin chemistry Co. Ltd. Lutrol F68 Micro was bought from BASF SE, Germany. Other reagents used in the experiment were all of analytical grade.

2.2. Animals

Kunming strain mice (25–28 g) were purchased from Center for New Drug Evaluation of Shandong University, Jinan, China. Mice were housed in groups of eight in cages with an aerating system. They were kept at a controlled temperature and humidity with a light/dark cycle of 12 h and they were fed with food and water. Mice in this experiment were acclimatized for at least 1 wk. All the procedures and care were conducted according to EU Directive 2010/63/EU for animal experiments.

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

Ripe *Z. jujube cv. Huanghetanzao* was pre-dried and washed with deionized water. Then, the pulp was stripped and dried at 45 °C in the oven over 12 h and crushed into powder. By using the ethanol refluxing method, some lipids and soluble materials were removed in an appropriate volume of 95% ethanol at 75 °C for twice (3 h each time). Subsequently, the ethanol-extracted residue was suspended in deionized water and sonicated at 50 °C for 15 min by using an ultrasonic cleaner. Then the mixture was cooled to ambient temperature and filtrated under vacuum to remove the insoluble substances such as cellulose. Anhydrous ethanol was slowly added to the filtrate until reaching the end concentration of 80% (v/v) to precipitate the macromolecules. Finally, the sediment was filtrated and freeze-dried to get HJP.

2.4. Quantification and identification of jujube monosaccharides and polysaccharides

The polysaccharides in (HJP) were quantified with the phenol–sulfuric acid method. Glucose calibration solutions were set up with seven different concentrations (10–70 μg/mL). According to the standard, the content of the polysaccharides (HJP) was calculated.

With a pre-column derivatization HPLC method, the monosaccharide components of HJP were analyzed. In brief, 30 mg sample of HJP was hydrolyzed to prepare monosaccharides by using sulfuric acid (H_2SO_4, 2 M) at 110 °C for 8 h. After neutralization with sodium hydroxide (NaOH), the solution (100 μL) was mixed with sodium hydroxide solution (150 μL, 0.3 M) and 1-phenyl-3-methyl-5-pyrazolone (PMP, 100 μL, 0.5 M) in methanol. Then the mixture was heated to 70 °C and reacted for 30 min. After being cooled to ambient temperature, the solution was neutralized with hydrochloric acid (HCl, 0.3 M). Subsequently, chloroform ($CHCl_3$) was utilized to extract the un-reacted PMP for three times. Finally, the aqueous phase was filtered through a 0.22-μm membrane for the HPLC analysis. 10 μL of the sample was injected and separated by an Elite Hypersil ODS2 column (4.6 mm × 250 mm, 5 μm) on an Agilent model 1200 HPLC system. Acetonitrile (C_2H_3N) and phosphate buffer (50 mM, pH 6.8) with the volume ratio of 22:78 were used as the mobile phase. The flow rate of elution was 1.0 mL/min and the column temperature was 25 °C. The wavelength for UV detection was set to be 250 nm. Standard monosaccharides were reacted with PMP using the same method for further qualitative and quantitative analysis.

2.5. The effects of HJP on acute hepatic damage induced by CCl_4

2.5.1. Therapeutic effects of HJP on the damage induced by CCl_4

After environmental adaptation for 1 wk, mice were divided randomly. Each group was assigned with eight mice. At first, CCl_4 were given *via* an intraperitoneal injection of 0.4% CCl_4 in peanut oil (v/v, 0.3 mL). Mice in the blank group only received the dosing vehicle only. An hour later, HJP or the commercial product silybin (INN, positive control) were intraperitoneally injected. For the HJP-treated groups, animals were given HJP with the dose of 100, 200, or 400 mg/kg body weight. In the positive control group, animals received silybin at a dose of 150 mg/kg. Mice in the blank and model groups only received the same volume of the physiological saline. All the animals were fasted but allowed to drink water as usual. 24 h after the intoxication for each mouse, eyeball was yanked to collect blood, and then cervical vertebra was dislocated mercifully. The samples of blood were placed at room temperature for 1 h, and then centrifuged at 4000 r/min for 15 min. The supernatants were drawn out as serum and stored at 4 °C.

To further confirm the therapeutic effects of HJP, the time–efficacy relationship was studied. After the injury by CCl_4, HJP (400 mg/kg) were intraperitoneally injected at 1 h, 3 h and 6 h. Mice in blank and model groups only received the physiological saline. 24 h after the intoxication, the serum was collected and stored as described above.

2.5.2. Prevention effects of HJP on the damage induced by CCl_4

Before the hepatic damage induced by CCl_4, HJP groups were treated with HJP (100 or 400 mg/kg, i.p.) once daily for 5