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Polyphenols from hawthorn peels and fleshes differently mitigate dyslipidemia, inflammation and oxidative stress in association with modulation of liver injury in high fructose diet-fed mice

Xiao Han, Wenfeng Li, Di Huang, Xingbin Yang*

Shaanxi Engineering Laboratory for Food Green Processing and Safety Control, College of Food Engineering and Nutritional Science, Shaanxi Normal University, Xi'an 710062, China

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ABSTRACT

Hawthorn ingestion is linked to health benefits due to the various polyphenols. The present study investigated the differential effects of polyphenols-enriched extracts from hawthorn fruit peels (HPP) and fleshes (HFP) against liver injury induced by high-fructose diet in mice. It was found that the main species of polyphenols in hawthorn was chlorogenic acid, epicatechin, rutin and hyperoside, and their contents in HPP were all higher than those in HFP. Administration of HPP was better than HFP to alleviate liver injury and hepatocyte apoptosis, reflected by the reduction of ALT, AST and ALP activities, as well as the ratio of Bax/Bcl-2 in mice. Meanwhile, HPP was also more effective than HFP to mitigate liver inflammation and oxidative stress by inhibiting inflammatory cytokine (TNF-α, IL-1 and IL-6) release, and elevating antioxidant enzyme activities and PPARα expression, while reducing Nrf-2 and ARE expression in mice. Interestingly, HPP-treated mice also showed the lower levels of TC, TG, LDL-C, VLDL-C and Apo-B, and the higher levels of HDL-C and Apo-A1 than HFP-treated mice via reducing FAS express. These results together with the histopathology of the liver with H&E and oil red O staining suggest that hawthorn fruit, especially its peel, is an excellent source of natural polyphenolic chemopreventive agents in the treatment of liver disorders.

1. Introduction

Fructose has been used as a major caloric sweetener found in soft drinks, canned fruits, juice beverages, baked goods and breakfast cereals in the past three decades, during which time the prevalence of obesity has increased [1]. The increasing evidence shows that high-fructose (HF) diet makes high risks of nonalcoholic fatty liver disease (NAFLD) [1], which is the most common hepatic manifestation of obesity, and may develop to non-alcoholic steatohepatitis, and eventually hepatocellular carcinoma [2].

Nevertheless, various epidemiological studies indicate that the increasing consumption of fruits and vegetables has health benefits due to the various polyphenols, particularly in the prevention of chronic diseases, such as liver damage, diabetes, Alzheimer disease and cancer [3,4]. Hawthorn, which is widely distributed in Asia, Europe and North America [5], contains a variety of polyphenols, and it has been widely used as foodstuffs or medicinal materials in China and the other countries [6]. Besides, Kao et al. have demonstrated that polyphenols of hawthorn fruits can present anti-inflammatory potential *in vitro* and *in vivo* [7], suggesting that it may play a role in hepatoprotection. However, it is not clear about the diversity of hepatoprotective polyphenols between peels and fleshes of the hawthorn fruit.

This study was therefore designed to evaluate the efficacy of hawthorn peel polyphenols (HPP) and flesh polyphenols (HFP) to mitigate liver injury in the mouse model induced by consumption of a high-fructose diet. For this purpose, alcoholic extracts from hawthorn peels and fleshes were prepared, and their phenolic compounds were identified by high performance liquid chromatography (HPLC). Importantly, the differential hepatoprotective effects of phenolic extracts between hawthorn fruit peels and fleshes were assessed and compared by determining the modification of inflammation, oxidative stress, dyslipidemia and histological observation in mice.

* Corresponding author. Tel.: +86 29 85310580; fax: +86 29 85310517.
E-mail address: xbyang@snnu.edu.cn (X. Yang).

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2. Materials and methods

2.1. Materials

Hawthorn fruits were purchased from the market in Xi'an of China. Standard products of caffeic acid, chlorogenic acid, quercetin, rutin, epicatechin and hyperoside were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Assay kits of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), total cholesterol (TC) and total triglyceride (TG) were purchased from Changchun Huili Biotechnology Co., Ltd. (Changchun, China). Detection kits of alkaline phosphatase (ALP), superoxide dismutase (T-SOD), malonaldehyde (MDA) and glutathione peroxidase (GSH-Px) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). ELISA kits of very low density lipoprotein cholesterol (VLDL-C), apoprotein A1 (Apo-A1), apoprotein B (Apo-B), tumor necrosis factor α (TNF-α), interleukin 1 (IL-1), interleukin 6 (IL-6) and fatty acid synthetase (FAS) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The primary antibodies against Bax, B-cell lymphoma-2 (Bcl-2), NF-E2-relatedfactor 2 (Nrf-2), antioxidant response element (ARE), peroxisome proliferator-activated receptor α (PPARα), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and the horseradish peroxidase (HPR)-conjugated goat anti-rabbit secondary antibody were provided by Proteintech Group, Inc. (Chicago, USA). BCA protein assay kit was purchased from Xi'an Heart Biological Technology Co., Ltd. (Xi'an, China). Food grade fructose was obtained from Senbo Biology Co., Ltd., (Xi'an, China). Deionized water was prepared using a Millipore Milli Q-Plus system (Millipore, Bedford, MA, USA).

2.2. Extraction of polyphenols in hawthorn fruits

The extraction was performed as described previously [8,9]. Firstly, the hawthorn fruit was trimmed both ends, hand peeled, removed the stones and kept in electric blast drying oven (TAISITE, Tianjin, China) with the temperature of 50 °C and air velocity of 0.5 m/s until drying completely, and then ground into fine powders separately before extraction. Dried peel or flesh samples (30 g) were extracted with 600 mL 80% aqueous ethanol at 70 °C for 60 min for three times. After centrifugation (10 min, 4000*g*), supernatants from each extraction were combined and cooled to room temperature. The ethanolic extract was concentrated with a vacuum rotary evaporator. Subsequently, the concentrated solution was extracted using ethyl acetate in a 1:2 ratio (v/v) for three times at room temperature. Finally, the solution of ethyl acetate was concentrated with a vacuum rotary evaporator and dried using a vacuum freeze drier. The polyphenolic extracts were named HPP and HFP from hawthorn peels and fleshes, respectively.

2.3. HPLC analysis of polyphenolic compounds

Phenolic composition of HPP or HFP was analyzed with a HPLC method [10]. The analysis was performed on a reversed-phase C_{18} column (4.6 mm i.d. × 250 mm, 5 μm, Inertsil ODS-SP, Japan) at 37 °C. The analysis of phenolic composition was carried out using a Shimadzu LC-2010A HPLC system which was equipped with an UV detector fixed at 280 nm, and an autosampler and Shimadzu Class-VP 6.1 workstation (SHIMADZU, Kyoto, Japan). Mobile phase A was ultrapure water containing 0.5% methanoic acid, and 80% acetonitrile with 20% methyl alcohol was used as mobile phase B. The gradient program was as follows: 0–5 min, 10% B hold; 5–15 min, linear gradient to 16% B; 15–40 min, linear gradient to 30% B; 40–55 min, linear gradient to 60% B; 55–70 min, linear gradient to 10% B; 70–75 min, 10% B hold. The flow rate was 1 mL/min and the injection volume was 10 μL.

2.4. Animals and animal care

All the mice were received humane care in compliance with institutional guidelines (XJYYLL-2015689). Healthy male Kunming mice (weight 18–22 g) were purchased from the Experimental Animal Center of the Fourth Military Medical University (Xi'an, China). The mice were housed at constant temperature (22 ± 2 °C) and humidity (55 ± 5%), and exposed to a 12 h light-dark cycle with unrestricted access to food and tap water.

After one week for adaptation of laboratory environment, they were divided into four groups averagely with 8 mice each. In normal control group, the mice were allowed free access to a standard rodent chow (40% corn flour, 26% wheat flour, 10% bran, 10% fish meal, 10% bean cake, 2% mineral, 1% coarse meal, and 1% vitamin) and tap water. Besides, the mice were administered intragastrically (ig, 0.4 mL) with physiological saline once daily during the experimental period. In high-fructose control group, the mice were allowed free access to the standard rodent chow and 30% high-fructose water, and the mice were also administered (ig, 0.4 mL) with physiological saline once daily. In polyphenols-treated groups, the mice were allowed free access to the standard rodent chow and 30% high-fructose water. Besides, the mice were administered (ig, 0.4 mL) with 400 mg/kg bw HPP or HFP once daily, respectively. We selected the middle and effective dose (400 mg/kg bw) as the gavage dosage according to the preliminary experiment results of dose-dependent test (200, 400 and 600 mg/kg bw) and our previous experiments [11]. All the administrations were at 9:00 a.m. once daily, and both the tap water and high-fructose water were renewed every other day. After 8 weeks, mice were fasted overnight, and then all the animals were fully anesthetized by the inhalation of isoflurane and weighed. The liver and fat were removed, weighed, quickly frozen at −80 °C. We calculated the hepatosomatic index (HI) through the records of body weight and liver weight according to the following formula: HI = liver weight (g)/body weight (g), and fat index (FI) through the records of body weight and fat weight according to the following formula: FI = fat weight (g)/body weight (g). All the experiments were performed according to the Guidelines of Experimental Animal Administration published by the State Committee of Science and Technology of People's Republic of China.

2.5. Biochemical assessment of the serum and liver

The serum was collected by centrifuging the blood samples at 3000*g* for 15 min and stored at 4 °C. Serum enzymatic activities of AST, ALT, and ALP, and the levels of VLDL-C, HDL-C, LDL-C, Apo-A1, and Apo-B were determined using commercial kits according to the instructions. Liver tissue was homogenized by an automatic homogenizer (F6/10-10G, FLUKO Equipment Shanghai Co. Ltd, Shanghai, China). In the preparation, 0.5 g of each liver tissue was homogenized in nine-fold (w/v) cold normal saline, and centrifuged at 1500*g* for 10 min [12]. The supernatant was collected and used for the measurements of TC, TG, FAS, IL-1, IL-6 and TNF-α by commercial kits. MDA was determined by the reaction with thiobarbituric acid (TBA), of which the amount of product (proportional to MDA present) is detected by a colorimetric assay [13]. Besides, SOD and GSH-Px were measured by the reaction with nitroblue tetrazolium (NBT) and 5, 5′-Dithio bis-(2-nitrobenzoic acid) (DTNB) depending on kits, respectively. Total protein contents in homogenates were determined by the method of Coomassie Brilliant Blue [14].