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Short communication

High molecular weight persimmon tannin is a potent hypolipidemic in high-cholesterol diet fed rats

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ABSTRACT

Aimed to elucidate whether high molecular weight persimmon tannin (HMWPT) is responsible for the hypolipidemic effect of consuming persimmon fruit, the effects of HMWPT on high-cholesterol diet fed rats were investigated. Male Sprague–Dawley rats were fed a 2% high-cholesterol diet and treated with different dosages of HMWPT or without HMWPT for 9 weeks, lipids and antioxidant profiles were examined and the morphology of livers was checked as well. The results indicated that HMWPT effectively reduced serum and hepatic triglyceride, total cholesterol and low density lipoprotein cholesterol while increased the serum and hepatic high density lipoprotein cholesterol ($p<0.05$). In addition, 100 mg/kg body weight per day of HMWPT treatment could significantly enhance the serum lecithin cholesterol acyl transferase (LCAT) activity and fecal bile acids excretion ($p<0.05$). Meanwhile, accumulation of hepatic lipid droplets and hepatic steatosis induced by high-cholesterol diet was inhibited markedly by HMWPT. Furthermore, high-cholesterol diet induced oxidative stress in rats but HMWPT significantly increased the decreased activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), elevated the lowered total anti-oxidation capability (T-AOC), ($p<0.05$), and decreased the raised malondialdehyde (MDA) levels ($p<0.05$) in serum or liver. These results suggested that HMWPT was responsible for the hypocholesterolemic effect of persimmon fruit and it might exert the hypolipidemic effect through stimulating serum LCAT activity, enhancing fecal bile acids excretion and improving antioxidant profile.

1. Introduction

Persimmon (*Diospyros kaki* L.) characterized by an abundant tannin content is cultivated widely in China, Korea and Japan. In recent years, the health benefits of persimmon fruit have attracted many researchers' attention (Giordani, Doumett, Nin, & Del Bubba, 2011). Dietary persimmon fruit (Gorinstein, Bartnikowska, Kulasek, Zemser, & Trakhtenberg, 1998; Matsumoto, Watanabe, Ohya, & Yokoyama, 2006; Matsumoto, Yokoyama, & Gato, 2008, 2010), peel and pulp (Gorinstein, Kulasek, et al., 1998) were reported to exert hypolipidemic properties in some hyperlipidemic animal models. However, most of these studies were conducted with whole persimmon fruit or persimmon peel or persimmon pulp as test materials. It was well known that persimmon fruit contained a large number of components such as condensed tannin, dietary fiber, carotenoids, gallic acid, catechins and flavonoids etc. (Akagi, Katayama-Ikegami, & Yonemori, 2011; Jo, Son, Shin, & Byun, 2003; Veberic, Jurhar, Mikulic-Petkovsek, Stampar, & Schmitzer, 2010). These components, such as dietary fiber, flavonoids and catechins, also showed hypolipidemic effects in some hyperlipidemic animal models (Ramulu, Giridharan, & Udayasekhararao, 2011). Therefore, whether persimmon tannin, especially the high molecular weight persimmon tannin which accounts for the majority of persimmon tannin, is responsible for the hypolipidemic effects of consuming persimmon is not clear. Although Gorinstein et al. (2000) compared the hypolipidemic and antioxidant effects of whole persimmon and phenol-free persimmon, but they used ethyl acetate to get rid of the phenols from persimmon fruit at room temperature. Due to the high polymerized structure, the highly polymerized tannin which accounts for the main component of

Abbreviations: AI, atherogenic index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BSA, bovine serum albumin; BW, body weight; CAT, catalase; DP, degree of polymerisation; ECG, (epi)catechin-3-O-gallate; EGCG, (epi)gallocatechin-3-O-gallate; FRAP, ferric reducing ability power; GSH-Px, glutathione peroxidase; HC, high cholesterol group; HC/HPT, high- cholesterol with high dose (100 mg/kg body weight) of persimmon tannin group; HC/LPT, high-cholesterol with low dose (25 mg/kg body weight) of persimmon tannin group; HC/MPT, high-cholesterol with moderate dose (50 mg/kg body weight) of persimmon tannin group; HDL-C, high-density lipoprotein cholesterol; HMG-CoAR, 3–hydroxy–3-methylglutaryl CoA reductase; HMWPT, high molecular weight persimmon tannin; LCAT, lecithin cholesterol acyl transferase; LDL-C, low-density lipoprotein cholesterol; LPS, lipase; MDA, malondialdehyde; NC, normal control group; NEFA, non-esterified fatty acids; NF-κB, nuclear factor-kappaB; PT40, persimmon tannin 40; ROS, reactive oxygen species; SOD, superoxide dismutase; T-AOC, total anti-oxidation capability; TBARS, thiobarbituric acid related substances; TC, total cholesterol; TG, triglyceride.

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persimmon tannin barely dissolves in ethyl acetate, and can hardly be removed under these conditions. Recently, Matsumoto et al. (2011) extracted a highly polymerized tannin from dried-young fruits of persimmon (*Diospyros kaki Hachiya*), and studied its bile acid-binding ability in vitro and in vivo, but its effects on the lipid metabolism have not been investigated. In addition, the chemical components and functional effects of persimmon tannin may differ significantly due to the different cultivars and producing areas (Nakatsubo et al., 2002). Our previous studies (Li, Leverence, et al., 2010) showed that high molecular weight persimmon condensed tannin (HMWPT) from a Chinese cultivar (*D. kaki Niuxin*) had a unique structure compared with other plant proanthocyanidins: It comprises an unusual flavonol myricetin terminal unit along with the more common flavan-3-ols catechin and epigallocatechin-3-*O*-gallate; it has a high prodelphinidin content (58–65%) and it has A-type interflavan linkages in addition to the more common B-type interflavan bonds. But so far, no work has been done to study whether the unique HMWPT is responsible for the hypolipidemic effects of persimmon fruit. Therefore, the present study was aimed to investigate whether the unique HMWPT exert hypolipidemic effect of on high-cholesterol diet induced hyperlipidemic rats, and the effects of HMWPT on the antioxidant profiles of high-cholesterol diet induced hyperlipidemic rats were also evaluated.

2. Materials and methods

2.1. Reagents

Commercial kits used for determination of superoxide dismutase (SOD), total anti-oxidation capability (T-AOC), glutathione peroxidase (GSH-Px), catalase (CAT), serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lipase (LPS) and serum non-esterified fatty acids (NEFA) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, PR China). ELISA kits for measuring serum lecithin cholesterol acyl transferase (LCAT) and hepatic 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoAR) activities were from Fengxiang Biotechnological (Shanghai, China). Commercial kits for determination of serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG) were from Shanghai Mind Bioengineering Co., Ltd. (Shanghai, China). All solvents and reagents were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and were of analytical grade.

2.2. Plant material and sample preparation

Mature and fully colored fruits of the astringent persimmon (*Diospyros kaki Niuxin*) were harvested in late November from an orchard in Shan'xi province (China). After harvest, fruits were held at 100 °C for about 5 min to inactivate polyphenol oxidase, and then stored deep frozen at −20 °C. According to our previous report (Gu et al., 2008), we extracted pulverized persimmon fruit thrice with methanol/HCl (1%, v/v) at 80 °C for 40 min. The concentrated extract solution was applied into a glass column (35×400 mm, i.d.), packed with AB-8 macroporous resin (Tianjin, China) equilibrated with water, eluted firstly with 500 ml deionized water at a flow rate of 3 ml/min to remove sugar and other soluble impurity. When the eluate was sugar-free, as indicated by lack of reactivity with phenol-sulfuric acid (DuBois, Gilles, Hamilton, Rebers, & Smith, 1956), 500 ml of 10% ethanol/water (v/v) was used to wash low molecular weight phenolic compounds. Absolute alcohol was used to elute the target tannins at a flow rate of 2 ml/min. After eluting, solvent was removed using a rotary evaporator under vacuum at 35 °C, and the residue was lyophilized. The content of total polyphenols in HMWPT was 98.7% on a mass basis by Folin–Denis method (Gahler, Otto, & Böhm, 2003) using gallic acid as a standard and the condensed tannins content was 93.4% on a mass basis by acid butanol assay (Porter, Hrstich, & Chan, 1985) with apple procyanidin dimers as a standard (Li, Trombley, Schmidt, & Hagerman, 2010). The mean degree of polymerization of PT40 was estimated to be 26 by thiolysis degradation combined HPLC-MS-MS analysis. The structural characterization and proposed structure were elucidated in our earlier papers (Li, Leverence, et al., 2010; Yang et al., 2012).

2.3. Experimental animals and diets

All experiments were performed in compliance with the Chinese legislation on the use and care of laboratory animals and were approved by the Huazhong Agricultural University of Science and Technology Committee on Animal Care and Use. Fifty male Sprague–Dawley rats, weighing 80–90 g, were purchased from the Tongji Medical College of Huazhong University of Science and Technology (Wuhan, China). The animals were housed in the temperature and humidity controlled room (temperature 24±2 °C and humidity 50±10%) with a 12 h light–dark cycle and given free access to diet and water.

After 1 week of acclimation to the laboratory, 10 rats were selected randomly as the normal control group (NC) which were fed basic diet, while the others were fed high-cholesterol diet (81.8% basic diet, 5% lard, 2% cholesterol, 0.2% sodium cholate, 5% full cream milk powder, 6% dried egg yolk) and randomly divided into 4 groups as follows: high cholesterol group (HC), high cholesterol + low, medium, or high dosage of HMWPT groups (designated as HC/LPT, HC/MPT, and HC/HPT respectively), each group consisting of 10 animals. Rats in HC/LPT, HC/MPT, and HC/HPT groups were administrated with HMWPT intragastrically (*i.g.*) daily at the doses of 25, 50 and 100 mg/kg.BW (body weight) respectively, for 9 weeks. At the same time, the NC group and the HC group rats were taken the same volume of physiological saline for 9 weeks by intragastric administration. Rats were given free access to food and water during the experimental period. Food consumption and body weight were recorded daily.

Feces were collected at the final 48 h and lyophilized for analysis. At the end of the experimental period, all animals were fasted 14 h before anesthetized with chloral hydrate and sacrificed. Blood samples were drawn from the ophthalmic venous plexus. After centrifugation (5000*g*, 15 min, 4 °C), the serum samples were collected and stored at −20 °C. Liver was excised, rinsed in ice-cold 9 g/L sodium chloride, gently blotted on filter paper, weighted and then stored at −20 °C.

2.4. Serum, liver and fecal lipids analysis

Serum and liver levels of TC, HDL-C, LDL-C, and TG were measured using commercially available enzyme kits (Shanghai Mind Bioengineering, Shanghai, China) with a UV-1700 spectrophotometer (Shimadzu, Japan). TC were measured by an enzymatic method based on the liberation of free cholesterol by cholesterol ester hydrolase and the free cholesterol produced is oxidized by cholesterol oxidase to cholest-4-en-3-one with the simultaneous production of hydrogen peroxide, which oxidatively couples with 4-aminoantipyrine and phenol in the presence of peroxidase to yield a chromogen with maximum absorption at 500 nm (Allain, Poon, Chan, Richmond, & Fu, 1974). HDL-C were assayed by the same enzymatic method based on specific precipitation of VLDL-cholesterol and LDL-cholesterol in the presence of magnesium ions (Castelli et al., 1977), and LDL-C were measured by specific precipitation of LDL-C with polyvinyl sulfat (Assmann, Jabs, Kohnert, Nolte, & Schriewer, 1984). The determination of TG was based on a peroxidase-coupled method (McGowan, Artiss, Strandbergh, & Zak, 1983). Serum NEFA levels were assayed using a commercially available kit (Nanjing Jiancheng Bioengineering Institute Nanjing, PR China), which was based on the chromogenic reaction of NEFA with copper reagents and diphenylcarbazide (Falholt, Lund, & Falholt, 1973). Liver tissue and feces (0.5 g) were subjected to lipid extraction with 10 mL of cold chloroform–methanol (2:1, v/v) by the method of Folch, Lees,