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Characterisation of polysaccharides from green tea of *Huangshan Maofeng* with antioxidant and hepatoprotective effects

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ABSTRACT

This study was to examine the hepatoprotective effects of polysaccharides from green tea of *Huangshan Maofeng* (HMTP) against CCl_4-induced oxidative damage in mice. HMTP is an acidic heteropolysaccharide with galactose (35.0%, mol.%), arabinose (28.9%) and galacturonic acid (11.3%) being the main monosaccharide components. HMTP (400 and 800 mg/kg·bw) administered orally daily for 14 days before CCl_4 administration significantly reduced the impact of CCl_4 toxicity on the serum markers of liver damage, alanine aminotransferase, aspartate aminotransferase, total-cholesterol and triglycerides. This method of HMTP administration also markedly restrained hepatic lipid peroxidation formation of malondialdehyde and 15-F_{2t} isoprostanes, and elevated the antioxidant levels of hepatic glutathione and superoxide dismutase. These results together with liver histopathology indicated that HMTP exhibited hepatoprotection against CCl_4-induced injury, which was found to be comparable to that of biphenyldicarboxylate. The hepatoprotective effects of HMTP may be due to both the inhibition of lipid peroxidation and the increase of antioxidant activity.

1. Introduction

The liver is a vital organ and has many important functions, including metabolism and detoxification of hepatotoxicants (Altas, Kizil, Kizil, Ketani, & Haris, 2011). In most cases, liver damage is a widespread pathology which involves oxidative stress and a progressive evolution from steatosis to chronic hepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma (Srivastava & Shivanandappa, 2010). Various xenobiotics are known to cause hepatotoxicity, one of which is carbon tetrachloride (CCl_4). CCl_4 is a classical hepatotoxin leading to hepatic diseases, and is generally used in animal models for inducing acute liver injury (Weber, Boll, & Stampfl, 2003). Reductive dehalogenation of CCl_4, by the P450 enzyme system in the endoplasmic reticulum, into highly reactive trichloromethyl-free radicals ($^{\cdot}CCl_3$ or $CCl_3OO^{\cdot}$) initiates the process of lipid peroxidation. This is considered to be the most important mechanism in the pathogenesis of liver damage induced by CCl_4 (Altas et al., 2011). CCl_4-induced damage is also able to change the antioxidant status of the tissues, which is manifested by abnormal histopathological alteration with steatosis, centrilobular necrosis and cirrhosis in the liver (Xu et al., 2010).

In recent years, there has been increasing interest in the use of active dietary ingredients for the prevention of liver diseases all over the world, which are believed to be harmless and free from adverse reactions (Samojlik, Lakic, Mimica-Dukic, Dakovic-Svajcer, & Bozin, 2010). Green tea (*Camellia sinensis*) has a long history, as ancient as 2000 years in Asian countries (Chen, Zhang, Qu, & Xie, 2007), and is popularly consumed because it retains a basic crude plant material, which is different from semifermented (oolong tea) and fermented (black tea and pu-erh tea) forms, and keeps much of its intrinsic bioactive ingredients (Yang et al., 2010a). The *Huangshan Maofeng* tea, a principal source of Chinese green tea, is grown in Huangshan (Anhui, China), known for short sunshine-hour, so that its fresh leaves are thick, fat, long shoots with a sweet smell. It is therefore claimed that drinking the green tea can promote health and alleviate the severity of many disorders (Tian et al., 2012). It is also well known that different studies on the tea yield different results, possibly due to their various geographical origins or the purification process (Nie & Xie, 2011). Given that, further studies on the *H. Maofeng* tea can be useful to clarify its physiological benefits and also provide a clue to substantiate its traditional dietary and therapeutic uses.

Traditionally, low-molecular weight flavonoids have been considered as the active ingredients of *H. Maofeng* tea (Wu et al., 2011). Recently, the water-soluble polysaccharides have also received much attention as they are the other main bioactive components found in tea, other than tea polyphenols (Wei, Liu,

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Xiao, & Wang, 2009), and more readily soak out into a tea infusion, in comparison with the flavonoids (Yang, Lv, Tian, & Zhao, 2010b). It has been reported that natural polysaccharides, which are found largely in living organisms, can be exploited as novel potential antioxidants, and the effects are associated with their origins and chemically constitutive characterisation (Chen et al., 2007; Monobe, Ema, Kato, & Maeda-Yamamoto, 2008). Some tea polysaccharides have also been reported to have a variety of significant biological activities, such as reducing blood sugar level, immunomodulation, anti-radiation, anti-blood coagulation, anti-oxidant, anticancer and hypoglycemic activities (Nie & Xie, 2011; Lee et al., 2006; Wang, Wei, & Jin, 2009; Xu, Ye, Sun, Tu, & Zeng, 2012). Despite these reports, the chemically compositional structure of the polysaccharides of *H. Maofeng* green tea and their hepatoprotective benefits are not fully understood.

Here, our work was to isolate the polysaccharides from Chinese green tea of *H. Maofeng* (HMTP), and to screen their antioxidant activity *in vitro*. Furthermore, the *in vivo* protective effects of HMTP on CCl_4-induced hepatic damage in mice were also investigated in detail. Moreover, the chemical characterisation of HMTP was identified and quantified by HPLC–UV.

2. Materials and methods

2.1. Materials and chemicals

The green tea of *H. Maofeng*, a geographically specific *C. sinensis*, was purchased from a local tea market, which was harvested from the Huangshan region of Anhui province, China. Voucher specimens of the plant materials were deposited at the Key Laboratory of the Ministry of Education for Medicinal Resource and Natural Pharmaceutical Chemistry, Shaanxi Normal University, 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 (BHT), 1,1-diphenyl-2-picrylhydrazyl (DPPH), potassium ferricyanide [$K_3Fe(CN)_6$], trichloroacetic acid (TCA), and trifluoroacetic acid (TFA) were purchased from Sigma (Sigma–Aldrich GmbH, Sternheim, Germany). Triethylamine (TEA) and 1-phenyl-3-methyl-5-pyrazolone (PMP) were the products of Merck (Darmstadt, Germany). Biphenyldicarboxylate pills (BP) were obtained from Zhengjiang Wanbang Pharmaceutical Co. (Tianjin, China). Assay kits for serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), total-cholesterol (TC) and triglycerides (TG) were the products of Huili of Biotechnology (Changchun, China). The commercially available diagnostic kits of glutathione (GSH), total-superoxide dismutase (T-SOD) and malonaldehyde (MDA) were obtained from the Jiancheng Institute of Biotechnology (Nanjing, China). The ELISA (enzyme-linked immunosorbent assay) kit of 15-F_{2t} isoprostanes (original name 8-iso-PGF2α) was obtained from the Lantu Biotech Co., Ltd. (Fujian, China). Deionised water was prepared using a Millipore Milli Q-Plus system (Millipore, Bedford, MA, USA).

2.2. Extraction and isolation of HMTP

The polysaccharides of *H. Maofeng* green tea were isolated by a procedure as described previously (Wang et al., 2012). Briefly, the dried tea (200 g) was crushed into powder by a disintegrator. The powder was soaked in 95% ethanol (1:6, w/v) refluxing at 60 °C for 4 h as an ungreased treatment. After the mixture was filtered, the residues were dried in air and then were extracted with a tenfold volume of water (1:10, w/v) at 80 °C for 4 h, and this extraction was repeated three times. The combined extraction solution was concentrated to 200 ml using a rotary evaporator under reduced pressure at 60 °C, and then was precipitated three times by adding threefold volume of 95% (v/v) ethanol at 4 °C for 24 h. The separated precipitate was completely dissolved in an appropriate volume of water, and intensively dialysed for 4 days against distilled water (cut-off Mw 10,000 Da) to remove the small molecular compounds (e.g. flavonoids or polyphenols). The retentate portion was deproteinised by a freeze–thaw process (FD-1, Henan Yuhua Instrument Co., China) for at least 10 times in a plastic bottle, followed by filtration. Finally, the solution was centrifuged at 2300*g* for 20 min to remove insoluble substance and the filtrate was lyophilised to yield the brownish water-soluble polysaccharides of HMTP.

2.3. Chemical analysis of HMTP

The total carbohydrate content of HMTP was determined by a modified phenol–H_2SO_4 colorimetric method, with glucose as a standard (Beena, Krishna, & Pattabiraman, 1984). The absorbance of five calibration solutions of glucose (10–60 μg/ml) was determined at 490 nm using a spectrophotometer, and a standard curve was drawn with absorbance as ordinate and concentration as abscissa, the regression equation was obtained, and the sugars of HMTP were determined by comparison with the calibration curve. The uronic acid content was assayed using the m-hydroxydiphenyl method, with a glucuronic acid standard (Bitter & Muir, 1962). In addition, proteins in the polysaccharides were quantified according to the Bradford method, with bovine serum albumin as standard (Wei, Li, & Tong, 1997).

Reverse-phase HPLC was employed to measure the monosaccharide composition of HMTP as our previous procedures (Wang et al., 2012). HMTP sample (20 mg), together with fucose (4 mM) as an internal standard, was hydrolysed with 2 ml of 3 M TFA at 95 °C for 8 h in a 5-ml sealed ampoule filled with nitrogen gas, which was then cooled down and centrifuged at 180*g* for 5 min. The supernate was transferred to a 5-ml micro-round-bottomed flask and was dried under reduced pressure before dissolving with 1 ml ultrapure water. 100 μl of different concentrations of the hydrolysed monosaccharide solutions were labeled with PMP by spiking with 300 μl of 0.3 M aqueous NaOH and 200 μl of 0.5 M PMP methanol solution. After incubation at 70 °C for 1 h, the mixture solution was cooled to room temperature and neutralised by adding 300 μl of 0.3 M aqueous HCl. The final solution was extracted with 1 ml $CHCl_3$, and the aqueous phase was passed through a 0.45 μm membrane filter for HPLC analysis.

The HPLC analysis of the compositional monosaccharides in HMTP was performed using reversed-phase chromatography on a C_{18} column (4.6 mm i.d. × 250 mm, 5 μm, Venusil, USA) at 30 °C, using a Shimadzu LC-2010A HPLC system equipped with an UV detector fixed at 250 nm, an autosampler and the Shimadzu Class-VP 6.1 workstation (SHIMADZU, Kyoto, Japan). Mobile phase A consisted of acetonitrile and mobile phase B was 3.3 mM KH_2PO_4–3.9 mM TEA buffer containing 10% acetonitrile using a gradient elution of 95–95–92–89% B by a linear decrease from 0–5–20–40 min. Elution was carried out at a flow rate of 1.0 ml/min. The injection volume was 10 μl.

2.4. Animals

Sixty Kunming mice (18–22 g) were purchased from the Experimental Animal Center of the Fourth Military Medical University (Xi'an, China), and 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). The animals were housed in cages, with free access to water and food, in a standardisation animal laboratory, maintained at a temperature of 18–25 °C with a 12/12 h light–dark cycles and a humidity of 60 ± 5%. The experimental