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Protective effects of the total saponins from *Rosa laevigata* Michx fruit against carbon tetrachloride-induced acute liver injury in mice

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ABSTRACT

Saponins are the major chemicals from *Rosa laevigata* Michx fruit. However, there have no papers to report the activities of the natural product against liver damage. In the present work, total saponins from *R. laevigata* Michx fruit (RLTS) with purity > 70% was produced and then the protective effects of it against CCl_4-induced acute liver injury in mice was tested. The results showed that RLTS decreased serum ALT and AST activities compared with model group, as well as the relative liver weight and histological findings. In addition, RLTS remarkably increased the levels of SOD, CAT, GSH-Px, GSH and decreased MDA, iNOS and NO levels in liver. Transmission electron microscopy and TUNEL assays showed that RLTS repaired fragmented DNA and mitochondrial change caused by CCl_4. Further investigation demonstrated that RLTS pretreatment down-regulated the protein expression of CYP2E1, ATF6, GRP78, EIF2, COX-2, NF-κB, p53, Caspase-3, Caspase-9, Cytokeratin 18 and the levels of MAPKs phosphorylation, up-regulated Bcl-2 expression, and markedly decreased the gene expression of TNF-α, IL-6, Fas/FasL and Bax. This is the first time to reveal the hepatoprotective effect of total saponins from *R. laevigata* Michx fruit, which should be developed as a new drug for treatment of live injury in future.

1. Introduction

Liver disease is a common cause of death in the world (Williams, 2006) and drug-induced hepatotoxicity is a frequent cause of liver injury (Kaplowitz, 2004). Carbon tetrachloride (CCl_4)-induced hepatic injury is commonly used as an experimental model for evaluating the hepatoprotective activities of drugs (Jamshidzadeh et al., 2005). CCl_4 is a strong hepatotoxin that induces excessive production of free radicals and causes cellular necrosis, oxidative stress and inflammation, all of which lead to acute liver injury and failure (Brattin et al., 1985; Weber et al., 2003).

Nowadays, more and more investigations have been focused on development of new drugs for treatment of liver damage, and traditional Chinese medicine (TCM) has been concerned because of its high efficiency and low toxicity (Li et al., 2005; Ma et al., 2009). Some natural products have been found as the potent agents for treatment of liver injury induced by chemicals (Fan et al., 2009; Hsu et al., 2010; Brito et al., 2012). Thus, it is reasonable to find effective natural products from medicinal plants for treatment of liver injury.

Rosa laevigata Michx is a well-known medicinal plant in China and its fruit is widely consumed as invigorator, paregoric and astringent. Some works have been reported that this herb can cure hyperpiesia, chronic cough and dermatologic disease, as well as inhibit experimental arterial sclerosis (Zhang et al., 2004). The chemicals including polysaccharose, flavonoids and saponins are considered to be the main active components of this plant (Zhao et al., 2003). According to the reports, the compounds including ursolic acid, oleanolic acid, β-sitosterol, daucosterol, hederagenin and 2α,3β,19α-trihydroxyolean-12-en-28-oic acid are the main saponins from *R. laevigata* Michx fruit (Wang et al., 2000; Wu et al., 2009; Yan et al., 2010), and their chemical structures are given in Fig. 1. Our previous studies have shown that the flavonoids from *R. laevigata* Michx fruit has powerful antioxidant, hypolipidemic and antithrombosis activities (Liu et al., 2010; Zhang et al., 2013a), the protective effects against paracetamol and CCl_4-induced liver injury as well as nonalcohol fatty liver disease (Liu et al., 2011; Zhang et al., 2013b, 2013c). However, there have no papers to report the activities of the total saponins from *R. laevigata* Michx fruit against liver injury.

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The aim of the present work was to study the protective effects of the total saponins from *R. laevigata* Michx fruit (RLTS) against CCl_4-induced liver injury, and then the possible mechanisms were also investigated.

2. Materials and methods

2.1. Chemicals and reagents

The standard of oleanolic acid with purity of over 98% was purchased from National Institutes for Food and Drug Control of China (Beijing, China). CCl_4 was purchased from Kaixing Chemical Industry Co., Ltd. (Tianjin, China). Silymarin was produced from Sigma Chemical Company (Milan, Italy). Detection kits including alanine transaminase (ALT), aspartate transaminase (AST), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione (GSH), malondialdehyde (MDA), inducible nitric oxide synthase (iNOS) and nitric oxide (NO) were purchased from Nanjing Jiancheng Institute of Biotechnology (Nanjing, China). Tissue Protein Extraction Kit was obtained from KeyGEN Biotech. Co., Ltd. (Nanjing, China). Enhanced Bicinchoninic Acid (BCA) Protein Assay Kit was supplied by Beyotime Institute of Biotechnology (Jiangsu, China). 4′,6′-Diamidino-2-phenylindole (DAPI), Tris and SDS were purchased from Sigma (St. Louis, MO, USA). In Situ Cell Death Detection Kit, POD was provided by Roche Diagnostics (Roche Diagnostics Gmbh, Mannheim, Germany). Hematoxylin, Histostain-TM-Plus Kit and 3,3′-Diaminobenzidine tetrahydrochloride (DAB) Substrate Kit were purchased from Zhongshan Golden Bridge Biotechnology (Beijing, China). RNAiso Plus, PrimeScript® RT reagent Kit with gDNA Eraser (Perfect Real Time) and SYBR® *Premix Ex Taq*™ II (Tli RNaseH Plus) were supplied by TaKaRa Biotechnology Co., Ltd. (Dalian, China).

2.2. Herbal material and extraction

R. laevigata Michx fruit was purchased from Yunnan Qiancaoyuan Pharmaceutical Company Co. Ltd. (Yunnan, China) and identified by Dr. Yunpeng Diao (College of Pharmacy, Dalian Medical University, Dalian, China). A voucher specimen (DLMU, JYZ-2012080426) was deposited in the Herbarium of College of Pharmacy, Dalian Medical University (Dalian, China). The fruit was dried and grounded into powders (20–40 mesh) and 1200 g powders were extracted with 75% aqueous ethanol for 2 times (2.0 h for each). After extraction, the solution was combined and evaporated to no ethanol under reduced pressure at 60 °C, and the residue (1200 ml) was produced. Then, 1200 ml water was added into the residue to produce the sample solution, which was used for subsequent macroporous resin column chromatography.

2.3. Determination of the content of total saponins

The content of the total saponins in the extract was determined by using the method described previously (Li et al., 2008) with slight modifications. Briefly, the extract (10 mg) was dissolved in 50 ml of methanol. After evaporation of the solution (1 ml), the dry residue was mixed with 0.5 ml of 5% vanillin-acetic acid solution and 1.5 ml of perchloric acid, and then the mixture was incubated at 70 °C for 15 min. After cooled down in an ice bath, the solution was mixed with glacial acetic acid to 10 ml, and the absorbance was measured at 546 nm with a UV–Vis spectrophotometer (U-3010, Hitachi, Japan). The content of total saponins was quantified based on the standard calibration curve of oleanolic acid ($Y = 54.745X - 0.0664$, $R^2 = 0.9982$).

2.4. Selecting suitable resin for cleaning-up

Five types of macroporous resins including D101, DM301, D4020, D318 and X-5 were purchased from Haiguang Chemical Factory (Tianjin, China). One gram of each dry resin was placed into an Erlenmeyer flask and 30 ml sample solution was also added. The flask was then shaken in a shaker (120 rpm) at 25 °C for 12 h. After reaching the adsorption equilibrium, the solutions were removed and the content of the total saponins was analyzed. After that, the resin was washed with water to remove the residual solvent, and then the desorption experiments were carried out. The flasks were shaken (120 rpm) for 6 h at 25 °C after adding 30 ml 80% aqueous ethanol and the desorption solutions were analyzed. The adsorption capacity and desorption ratio of each resin for RLTS were calculated based on the following formula (1) and (2), respectively.

$$\text{Adsorption capacity} = \frac{V_0 \times (C_0 - C_e)}{W} \quad (1)$$

$$\text{Desorption ratio} = \frac{V_d C_d}{V_0 \times (C_0 - C_e)} \quad (2)$$

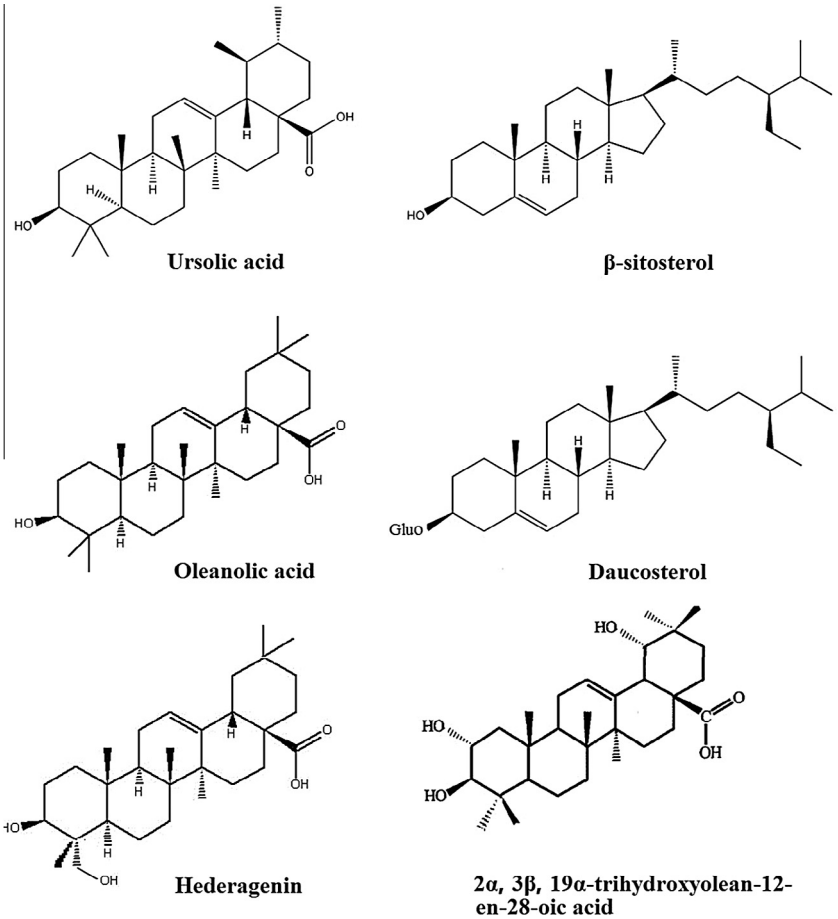

Fig. 1. The chemical structures of some main saponins from *R. laevigata* Michx.