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Original article

Hepatoprotective standardized EtOH–water extract from the seeds of *Fraxinus rhynchophylla* Hance

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ABSTRACT

Fraxinus rhynchophylla Hance (Oleaceae), its stem barks are known as *Cortex fraxini* (秦皮 qín pí) listed in Chinese Pharmacopoeia. Phytochemical study has indicated that methanol extracts from Qinpi has protective effect on acute liver injury. The present study investigates the hepatoprotective activity of EtOH–water extract from the seeds of *F. rhynchophylla* Hance against carbon tetrachloride-induced liver injury in mice. The EtOH–water extract significantly alleviated liver damage as indicated by the decreased levels of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), the malondialdehyde (MDA) content, and increased the levels of superoxide dismutase (SOD), glutathione (GSH) and glutathione peroxidase (GSH-Px), and reduced the pathological tissue injury induced by CCl_4. Quantitative analysis of seven major constituents (**1**–**7**) in EtOH–water extract (EWE) was developed by high performance liquid chromatography-diode-array detector (HPLC-DAD). The current research indicates that the EWE from the seeds of *F. rhynchophylla* Hance decreased liver index, inhibited the increase of serum aminotransferase induced by CCl_4, and decreased hepatic MDA content, SOD and GSH-Px activities. These results suggested that the pretreatment with EWE protected mice against CCl_4-induced liver injuries. Based on the results, the EtOH–water extract from the seeds of *F. rhynchophylla* Hance is efficacious for prevention and treatment of CCl_4-induced hepatic injury in mice. Secoiridoid and tyrosol glucosides might be the active ingredients responsible for the biological and pharmacological activities of hepatoprotection.

1. Introduction

The liver plays an important role in human metabolism and detoxification of endogenous and exogenous chemicals.[1] Liver injuries or dysfunctions have been recognized as serious health problem. Especially acute and chronic liver injuries resulted from the exposure to toxic chemicals, drugs, and virus infiltration from ingestion or infection, have gained more attention in recent years.[2–4] Corticosteroids and interferon has been used for the treatment of hepatic diseases, however, these synthetic chemical drugs are not well accepted by patients due to limited therapeutic efficacy and serious complications.[5] Therefore, more effective complementary and therapeutic drugs with low or no side-effects are needed for the treatment of liver diseases.[6–9] In recent years, some effective and safe dietary ingredients for liver-protection have been isolated from traditional medicinal plants, such as glycyrrhizin,[10] curcumin,[11] resveratrol,[12] as well as silybin and silymarin.[13,14] *Fraxinus rhynchophylla* Hance (Oleaceae) is a commonly used Chinese traditional medicinal plant, mainly distributed in China and Korea.[15] Its stem bark also known as *Cortex fraxini* (Qinpi) is Chinese herbal drug for treating diseases such as acute conjunctivitis and psoriasis; arresting discharges; curing chronic bronchitis; and bacillary dysentery, diuretic, antirheumatic, analgesic, antiperspiratory effects, and enhancing eyesight.[16] Phytochemical study has indicated that methanol extracts from Qinpi has protective effect on acute liver injury.[17] Many natural products such

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as secoiridoid glucosides, coumarins, lignans, sesquilignans, and coumarinolignans have been identified from this plant.[18–23] Recently, several pharmacological activities of phytochemical constituents isolated from the barks and leaves of *F. rhynchophylla* have been carried out, including anti-diabetes effects,[24–28] anti-*Toxoplasma gondii* effects,[29] including antioxidant enzymes,[30] inhibiting amyloid-β-induced neuronal cell damage,[31] and inhibiting nitric oxide synthesis activities.[32] Hydrangeside B, along with other secoiridoid glucosides showed hepatoprotective activities against DL-galactosamine-induced toxicity in human hepatocyte HL-7702 (HL-7702) cells.[33] Secoiridoid glycoside, oleuropein, showed anti-hepatitis B virus (HBV) activity and effectively blocked hepatitis B surface antigen (HBsAg) secretion with an IC_{50} of 23.2 μg/mL in HepG2.2.15 cells with no significant cytotoxicity.[34] The hepatoprotective activity of oleuropein against carbon tetrachloride (CCl_4)-induced liver damage in mice was achieved through the NF-E2-related factor 2-mediated induction of heme oxygenase-1.[35] Recently, Peng et al.[36] investigated the effect of *Fraxinus rhynchophylla* ethanol extract (FR_{EtOH}) on liver fibrosis induced by CCl_4 in rats. However, the hepatoprotective activity of the seeds of *F. rhynchophylla* Hance has not been evaluated so far.

The present study aimed to evaluate the hepatoprotective activity of EtOH–water extract from the seeds of *F. rhynchophylla* Hance employing a widely used CCl_4-induced liver damage model in mice and quantitative analysis of six secoiridoid glucosides (**1**–**6**) and one tyrosol glucoside (**7**) by high performance liquid chromatography-diode-array detector (HPLC-DAD) method.

2. Materials and methods

2.1. Chemicals and reagents

CH_3OH (HPLC grade), CH_3CH_2OH (HPLC grade), and CH_3CN (HPLC grade) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other organic solvents used in the current study, such as CH_3OH, ethyl acetate (EtOAc), acetone, and chloroform ($CHCl_3$) were of analytical grade. They are commercially available from Hengxing Chemical Reagent Co., Ltd. (Tianjin, China). Chemical standards of oleoside dimethyl ester (**1**), ligstroside (**2**), nuzhenide (**3**), 10-hydroxoleoside dimethyl ester (**4**), GI3 (**5**), GI5 (**6**), and salidroside (**7**) were prepared in our laboratory. The purity of each compound was >98%, determined by HPLC analysis. The chemical structures of these reference compounds are shown in Fig. 1.

2.2. Materials

The seeds of *F. rhynchophylla* Hance were provided in August 2013 from Baoji City, Shaanxi province, China. The herbariums of *F. rhynchophylla* Hance (FRH001) were deposited in Room 612, Department of Pharmaceutical Engineering, College of Chemical Engineering, Northwest University.

2.3. HPLC analysis conditions

HPLC analysis was performed on an Agilent 1260 separation module connected to a G1315D DAD detector using a Synergi 4u Hydro-RP 80R column (250 × 4.6 mm, 4 μm, 100 Å) with a flow rate of 1.0 mL/min. Solvent system: 0 min: 95% A (1% phosphoric acid) and 5% B (acetonitrile), 2 min: 95% A (1% phosphoric acid) and 5% B (acetonitrile), 5 min: 80% A (1% phosphoric acid) and 20% B (acetonitrile), 25 min: 75% A (1% phosphoric acid) and 25% B (acetonitrile), 27 min: 95% A (1% phosphoric acid) and 5% B (acetonitrile), 30 min: 95% A (1% phosphoric acid) and 5% B (acetonitrile). At the end of the run, 100% of acetonitrile was allowed to flush the column for 10 min, and an additional 10 min of post run time was set to allow for equilibration of the column with the starting eluant. The UV detector was operating at 230 nm, and the column temperature was maintained at 30 °C.

2.4. Calibration curves

Methanol stock solutions containing the seven standard compounds **1**–**7** were prepared and diluted to five different final concentrations. A calibration curve was constructed for each of the compounds by plotting peak areas versus compound concentrations.

2.5. Preparation of seeds of F. rhynchophylla Hance extract

The 5 kg air-dried seeds of *F. rhynchophylla* Hance were percolated twice with absolute ethyl alcohol at room temperature. The ethyl alcohol was evaporated under vacuum. The herb residue was then percolated twice with water at room temperature and made the SFR-water extracts. Finally, mixed the above two extracts, and the *in vivo* bioactivity study of seeds of *F. rhynchophylla* Hance was carried on by this sample. The HPLC spectrum of the extract is shown in Fig. 2.

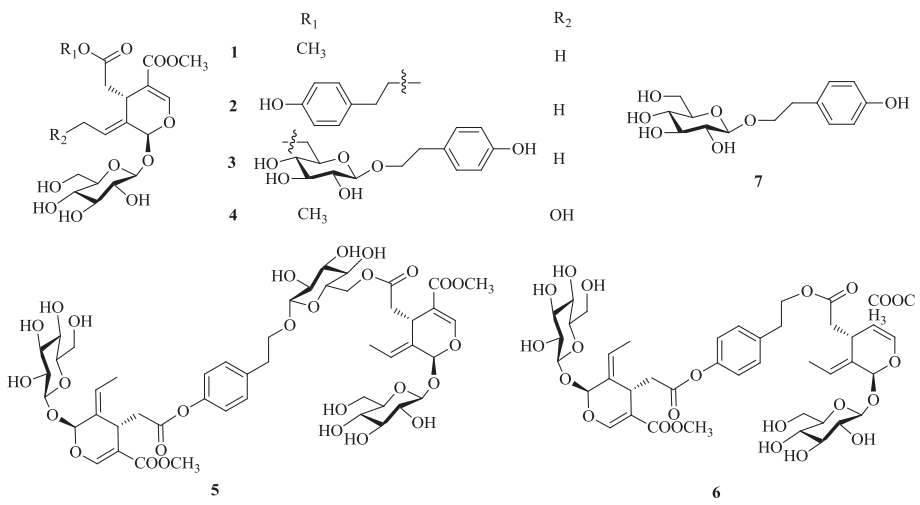

Fig. 1. Structures of compounds **1**–**7**.

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