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The protective effect of total phenolics from *Oenanthe Javanica* on acute liver failure induced by D-galactosamine

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ABSTRACT

Ethnopharmacology relevance: Water dropwort [*Oenanthe javanica* (*O. javanica*)] is an aquatic perennial herb cultivated in East Asian countries. It has been popularly used in traditional Chinese medicine which is beneficial for the treatment of many diseases, including jaundice and various types of chronic and acute hepatitis. In the present study, we investigated the hepatoprotective effect of total phenolics from *O. javanica* (TPOJ) against D-galactosamine (D-GalN) induced liver injury in mice.

Material and methods: The hepatoprotective activity of TPOJ (125, 250 and 500 mg/kg) was investigated on D-GalN (800 mg/kg)-induced liver damages in mice. Blood and liver were collected for biochemical and microscopic analysis. RT-PCR was used to determine the changes in hepatic nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) expression. Protein levels of iNOS, COX-2, superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) were determined by western blotting.

Results: In the animal studies, TPOJ could improve the survival of acute liver failure model significantly and prevente the D-GalN-induced elevation of the serum enzymatic markers and nonenzymatic markers levels significantly. Meanwhile, TPOJ-treatment decreased the malondialdehyde (MDA) level and elevated the content of glutathione (GSH) in the liver as compared to those in the D-GalN group. Hepatic activities and protein expressions of antioxidative enzymes, including SOD, GPx, and CAT were enhanced dose dependently with TPOJ. At the same time, application of TPOJ effectively suppressed the D-GalN-induced proinflammatory mRNA and protein expression of iNOS and COX-2. Subsequently, the serum levels of proinflammatory mediators, nitric oxide (NO) and prostaglandin E_2 (PGE_2) were reduced. Additionally, histological analyses also showed that TPOJ reduced the extent of liver lesions induced by D-GalN.

Conclusion: Our investigation demonstrated the hepatoprotective activity of TPOJ and revealed that TPOJ attributed its significance in the traditional use for treating liver diseases.

1. Introduction

Liver diseases are mainly classified into viral hepatitis, nonalcoholic fatty liver, alcoholic liver disease, autoimmune liver disease, schistosomiasis liver disease, drug-induced liver injury, hereditary liver disease, liver cirrhosis due to various causes and diverse liver tumors. Medicinal plants used in traditional Chinese medicine (TCM) have been widely used to treat liver diseases because of their ability to protect hepatocytes, inhibit hepatic inflammation and reduce fibrosis in the liver (Zhao et al., 2014). Although there is lack of evidence for clarification of their typical mechanisms, different from pharmaceutical chemicals, it is still widely accepted by people from East Asia and beginning to be accepted by the rest of the world.

Water dropwort [*Oenanthe javanica* (*O. javanica*)] is an aquatic perennial herb cultivated in East Asian countries such as China, Korea, and Japan, and belongs to *Oenanthe* genus in Apiaceae family. *O. javanica* with abundant vitamins and minerals is not only

Abbreviations: TPOJ, Total Phenolics from *Oenanthe Javanica*; TCM, Traditional Chinese Medicine; D-GalN, D-Galactosamine; iNOS, Nitric Oxide Synthase; COX-2, Cyclooxygenase-2; SOD, Superoxide Dismutase; GPx, Glutathione Peroxidase; CAT, Catalase; MDA, Malondialdehyde; GSH, Glutathione; NO, Nitric Oxide; PGE_2, Prostaglandin E_2; HBV, Hepatitis B Virus; AST, Aspartate Aminotransferase; ALT, Alanine Aminotransferase; ALP, Alkaline Phosphatase; TBIL, Total Bilirubin; GGT, Gamma-Glutamyltransferase; GAPDH, Glyceraldehyde 3-Phosphate Dehydrogenase; ROS, Reactive Oxygen Species

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consumed as a spicy vegetable especially in early spring because of its distinctive aroma and taste, but also has been used as a medicinal agent for hundreds of years. In TCM, *O. javanica* is recommended as a treatment for jaundice, various types of chronic and acute hepatitis, hypertension, fever, abdominal pain, leucorrhea, mumps, and urinary infections (Huang et al., 2001). The constituents of *O. javanica* were stated to consist of volatite oils, phthalic acid ester, amino acids, flavonoids, and phenolics (Zhang et al., 2012). Several studies indicated pharmacological benefits of *O. javanica*, including hepatoprotective, antithrombotic, neuroprotective, and anticancer activities. Our researches have already shown that *O. javanica* extracts had anti-diabetic activity by promoting insulin release from Langerhans β-cells due to flavonoids (Yang et al., 2000) and had anti-hepatitis B virus (HBV) activity on different HBV animal models such as rat, mouse and duck due to total phenolics (Han et al., 2008).

The search for hepatoprotective agents from natural herbal medicines is an area of considerable interest (Ram, 2001). Korea researchers isolated three major flavonoids including isorhamnetin sulfate, hyperin and persicarin from the leaves and stems of *O. javanica* and found persicarin exhibiting high hepatoprotective activity against the hepatic lipid peroxidation in bromobenzene or acetaminophen-treated rats (Park et al., 1996). Enhanced enzyme activity for alcohol detoxification by persicarin was also recognized in ethanol treated rats (Kim et al., 2009). Furthermore, *O. javanica* extracts, naturally fermented by steeping with oligosaccharides, had anti-proliferative effects on HepG2 cells (Kim et al., 2011). And recently, Yang et al. (2014) demonstrated the protective effects of fermented field *O. javanica* extracts on tert-butyl hydroperoxide (t-BHP)-induced hepatotoxicity in HepG2 cells and carbon tetrachloride (CCl_4)-induced liver damage in rats, thus indicating the potential of fermented field *O. javanica* extract as a therapeutic for acute liver diseases. However, no study has yet been performed on the hepatoprotective effect of total phenolics extracted from *O. javanica* (TPOJ), which was considered as one of the main components of *O. javanica*. Under the foundation of our previous studies (Han et al., 2008; Tian et al., 2010), the present research was undertaken to identify the hepatoprotective effect of TPOJ on D-galactosamine (D-GalN)-induced acute liver failure model in mice. We also investigated the underlying mechanisms based on the therapeutic role of TPOJ on the amelioration of oxidative stress and inflammation.

2. Materials and methods

2.1. Preparation of TPOJ

O. javanica was collected from Kai Yuan, Liao Ning Province in China, in spring 2013 and identified by Professor Hui-zhong Xiao, Department of Phytochemistry, Yanbian Medical University, China. A voucher specimen was deposited to their herbarium with the registration number 2013-087 in the Department of Phytochemistry, Yanbian Medical University (Yanbian, China). TPOJ was extracted according to a previously described method (Han et al., 2008). Folin-Phenol method.

was applied to the determination of total phenolics content using gallic acid as calibration standard. The result was expressed as mg of gallic acid equivalent (GAE) per g of TPOJ (mg GAE/g TPOJ) using equations that were obtained from a standard gallic acid graph. The result of quantification indicated that TPOJ had high content of phenolic compounds that made up TPOJ (523.7 ± 23.4 mg GAE/g TPOJ).

2.2. HPLC analysis of TPOJ

In addition, the HPLC analysis of TPOJ was performed on an Agilent 1100 HPLC.

instrument (Agilent, USA) equipped with a Diamonsil C_{18} column (250 mm × 4.6 mm, 5 μm) (Tian et al., 2010). The gradient elution was adopted with A: acetonitrile-methanol (10:1) and B: 0.4% phosphoric acid and was delivered at a flow rate of 1 mL/min. The linear gradient was 10% A to 90% A in 30 min. The UV detector was set at 325 nm and the column temperature was maintained at 25 °C. The sample injection volume was 20 μL. Chlorogenic acid, caffeic acid, 5-*O*-caffeoylquinic acid, butanedioic acid and gallic acid were identified by HPLC analysis. The five detected phenolic components probably were mainly bioactive compounds contributing to the hepatoprotective effect of TPOJ.

2.3. Animals

Male Kun-Ming mice were supplied by the Animal Raising Center of the Academy of Military Medical Sciences (Beijing, China). The animals were housed in stainless-steel cages in a room with controlled temperature (25 ± 1 °C) and humidity ($65 \pm 5\%$) and a 12-h light/dark cycle. The animals were fed with standard diet and had free access to water. All procedures involving animals and their care were carried out according to the guidelines of the Institutional Ethical Committee for Care and Use of Laboratory Animal of Academy of Military Medical Sciences in accordance with the governmental guidelines on animal experimentation, National Institutes of Health "Principles of Laboratory Animal Care".

2.4. Animal treatment and hepatoprotective assessments

The hepatoprotective activity of TPOJ in vivo was studied using a mouse model of D-GalN-induced hepatotoxicity. D-GalN, inducing oxidative stress, apoptosis and necrosis in both liver tissue and cultured hepatocytes, is a highly selective hepatotoxin frequently used in the experiments (Moravcova et al., 2014; Shin et al., 2014). After seven days of acclimatization, the male Kun-Ming mice, weighing 18–22 g, were randomly divided into six groups consisting of twelve mice per group. Normal and model control groups received 0.5% sodium carboxymethylcellulose (CMC-Na) solution at a dose of 10 mL/kg. Drug control group received silymarin (Tianjin Institute of Pharmaceutical Research, Tianjin, China) at a dose of 200 mg/kg, since it was reported to have a protective effect on the plasma membrane of hepatocytes (Madrigal-Santillán et al., 2014). Experimental drug groups received TPOJ at doses of 125, 250, and 500 mg/kg, representative of low, medium, and high dosage, respectively. The drugs were administered orally to the groups of mice, respectively, once per day for 7 days. One hour after the last administration, liver damage was induced in mice of model and drug groups by intraperitoneal injection of D-GalN at a dose of 800 mg/kg. At 12 h after D-GalN administration, all mice in each group were anesthetized with ether and then sacrificed. Blood was collected from the abdominal aorta. Liver tissues were immediately removed and quickly frozen and stored at −80 °C until further analysis.

Another sixty mice were divided into six groups consisting of ten mice per group for observation of survival rate. The treatments were the same as mentioned above. The observation was begun with the time of D-GalN injection, while the endpoint was set at 120 h after the injection.

2.5. Measurement of serum biochemical parameters

The blood samples were centrifuged (2500 rpm × 15 min, 4 °C)