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Hepatoprotective effect and its possible mechanism of *Coptidis rhizoma* aqueous extract on carbon tetrachloride-induced chronic liver hepatotoxicity in rats

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ABSTRACT

Ethnopharmacological relevance: Coptidis rhizoma is traditionally used for heat-clearing and toxic-scavenging and it belongs to liver meridian in Chinese medicine practice. Clinically, *Coptidis rhizoma* can be used for hepatic and biliary disorders, yet details in the therapies of liver diseases and underlying mechanism(s) remain unclear. Our previous study demonstrated that *Coptidis rhizoma* aqueous extract (CRAE) against CCl_4-induced acute liver damage was related to antioxidant property. In the present study, the protection of CRAE on chronic liver damage induced by carbon tetrachloride (CCl_4) in rats and its related mechanism were explored.

Materials and methods: The CCl_4-induced chronic liver damage model was established, and CRAE's protective effect was examined. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity, serum and liver superoxide dismutase (SOD) activity were then measured. The histological changes were observed under microscopy and then computed in numerical score. The normal or damaged cells were isolated and related signaling pathway was evaluated.

Result: Serum AST and ALT activities were significantly decreased in rats treated with different doses of CRAE, indicating its protective effect against CCl_4-induced chronic liver damage. Observation on serum SOD activity revealed that CRAE might act as an anti-oxidant agent against CCl_4-induced chronic oxide stress. Histological study supported these observations. Erk1/2 inhibition may take part into CRAE's effect on preventing hepatocyte from apoptosis when exposed to oxidative stress.

Conclusion: CRAE showed protective effect against CCl_4-induced chronic liver damage in rats and its potential as an agent in the treatment of chronic liver diseases by protecting hepatocyte from injury.

1. Introduction

Hepatocellular carcinoma (HCC), amounting for 80–90% of liver cancer, has nowadays become one of the most common and prevalent human malignancies in the world (Gramantieri et al., 2007; Budhu et al., 2008). One of the risky factors that can induce HCC is extended and chronic liver damage, the process causes demolishment of the normal liver blood system, leading to short supply of blood circulation in liver cells and consequently inducing hypoxia, the condition that normal cells are exposed to tremendous oxidative stress (Wu et al., 2007). One of the effective treatments in suppressing HCC is to control progress of chronic liver damage (Feng et al., 2009b).

Recent years, herbal medicines as a resource for liver diseases have been attracted by the world wide scientists (Schuppan et al., 1999; Feng et al., 2009b; Seeff et al., 2001), among which *Coptidis rhizoma* (CR, Huanglian in Chinese) is one of the potential herbs (Feng et al., 2009b; Ye et al., 2009). CR was a Chinese medicinal herb that is widely used for clearing heat and scavenging toxics during thousand years of clinical utilization. CR comprises various kinds of chemicals including berberine, palmatine and jatrorrhizine (Deng et al., 2008), among which berberine is the major ingredient representing a variety of bioactivities. Extensive studies in recent years have displayed that CR has various kinds of bioactivities including antibacterial, antiviral, antiinflammatory, antineoplastic, antihypertensive, antioxidative, antihyperglycemic and cholesterol-lowering effects (Fukutake et al., 1998; Li et al., 2000; Chang et al., 2001; Sanae et al., 2001; Yokozawa et al., 2003,

Abbreviations: HCC, hepatocellular carcinoma; CRAE, *Coptidis rhizoma* aqueous extract; CCl_4, carbon tetrachloride; AST, aspartate aminotransferase; ALT, alanine aminotransferase; SOD, superoxide dismutase; BW, body weight; SD rats, Sprague-Dawley rats; H&E staining, hematoxylin and eosin staining.

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2004; Choi et al., 2007; Kim et al., 2008). Our clinical study showed that CR had a promising potential as a drug for treatment of liver diseases, such as liver fibrosis and cancer (Feng et al., 2008). Moreover, it has been demonstrated in our previous studies that CRAE and its active compound, berberine exhibited potent protective effect on CCl_4-induced acute liver damage in rats (Ye et al., 2009; Feng et al., 2010). However, no observation and investigation has been proceeded to study CRAE's effect on chronic liver damage and its underlying mechanism.

In this study, CCl_4-induced chronic liver injury in rats was introduced as an animal model to investigate the protective effect of CRAE. HPLC was used in this study to analyze the chemical composition of CRAE. Serum AST and ALT was measured to observe the liver function in different treatment groups, and serum and tissue SOD activities were determined. Histological study was conducted to observe the morphological changes among different groups. To elucidate the underlying mechanism of CRAE's protection against chronic liver damage induced by CCl_4, rat hepatocyte was isolated and the apoptosis was evaluated by DNA fragmentation and caspase-3 activity. The related signaling transduction was also examined in this study.

2. Materials and methods

2.1. Chemicals

Berberine hydrochloride and palmatine hydrochloride were purchased from Sigma (Sigma–Aldrich, USA). Jatrorrhizine hydrochloride was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Magnolflorine chloride was purchased from Tauto Biotech (Shanghai, China). Coptisine was purchased from Chromadex (USA). Carbon tetrachloride (CCl_4) and liquid paraffin were purchased from Sigma (Sigma–Aldrich, USA).

2.2. Herbs and sample preparation

CR was collected in the GAP authorized field for Huanglian cultivation in Shizu county of Chongqing city of People Republic of China. Plants were cleaned under distilled water, dried and cut into small pieces. The roots of *Coptidis* were first boiled in distilled water at 100 °C for 1 h. The solution was percolated through filter paper (Whatman, pleated filter grade 597 1/2, 4–7 μm) and then sterilized by filtration through a 0.2 μm pore filter (Minisart®-plus, Sartorius). The collected extraction was evaporated to dryness by vacuum at temperature. The dry extract powder obtained is stored in −20 °C freezer and used in following experiments.

2.3. Phytochemical analysis

High performance liquid chromatography (HPLC) was introduced to analyze the active components in CRAE. The phytochemical analysis was performed under the following conditions: Reserve C18 column (Symmetry®, 250 mm × 4.0 mm, 5 μm) was used as solid phase; elution was performed using acetonitrile–25 mM potassium dihydrogen phosphate (23:77) as mobile phase. Detecting wavelength and flow rate was 254 nm and 1.0 ml/min respectively. The analysis was performed under room temperature.

2.4. Animals

Male SD rats weighing about 200 g were used. All animals were fed a standard diet ad libitum and housed at the temperature of 20–25 °C under a 12 h light/dark cycle throughout the experiment. The rats were randomly assigned into different groups: normal group, control group, CRAE low dose (400 mg/kg body weight, BW) group, CRAE medium dose (600 mg/kg BW) group, CRAE high dose (800 mg/kg BW) group and berbeine (120 mg/kg BW) group. All animals received humane care and study protocols complied with the guidelines of the animal center of the University of Hong Kong.

2.5. Animal treatment

Control group was given an intraperitoneal injection of CCl_4 at 1 ml/kg per rat (diluted 1:1 in liquid paraffin. The following is the same) twice a week for 8 weeks. The dose and duration were adopted from the methods of previous studies (Luo et al., 2004; Chung et al., 2005). CRAE treatment groups were given an intraperitoneal injection of CCl_4 at 1 ml/kg per rat twice a week for 8 weeks, and were given CRAE once per day at the dose of 400, 600 and 800 mg/kg BW during the same period. Berberine treatment group received daily oral administration of BW 120 mg/kg berberine. Normal group was given an intraperitoneal injection of liquid paraffin at 1 ml/kg per rat twice a week for 8 weeks, and was given double-distilled water orally once per day in the same period. At the end of the period, the rats were anesthetized with ether, blood samples were collected by cardiac puncture and serum was obtained by centrifugation (3000 rpm, 12 min). Liver tissues were washed quickly *in situ* with ice-cold isotonic saline.

2.6. Biochemical assay and histological study

Serum was collected as mentioned above. ALT and AST activities were then determined under the manufacturer's instruction (Biovision US). ALT and AST activities were reported in terms of units per liter (U/L). Serum was collected and the SOD activity was measured using the manufacturer's instruction (Biovision US).

Liver tissues were collected from animals in different groups and were fixed in 10% buffered formaldehyde solution for at least 24 h. The paraffin sections were then prepared (Automatic Tissue Processor, Lipshaw) and cut into 5 μm thick sections by a Leica RM 2016 rotary microtome (Leica Instruments Ltd., Shanghai, China). The sections were stained with hematoxylin and eosin staining (H&E staining) and then mounted with Canada balsam (Sigma, USA). The degree of liver damage was examined under the microscope (Leica Microsystems Digital Imaging, Germany). The images were taken using Leica DFC 280 CCD camera at original magnification of 10 × 10. Chronic liver injury was then evaluated by grading the liver sections numerically to assess their histological features. Vacuolation, nuclei, hepatocyte necrosis, inflammatory cell infiltration and central vein and portal triad were used as criteria, and a combined score of histological features was given for each liver section. The parameters were graded from score 0 to 6, with 0 indicating no abnormality, 1–2 indicating mild injury, 3–4 indicating moderate injury and 5–6 with severe liver injury (Wang et al., 2008).

2.7. Cells

The rat hepatocyte was isolated by *in situ* perfusion method as previously described (Seglen, 1973). Isolated cells were cultured in Williams' medium E (WME, Sigma–Aldrich, USA) supplemented with 10% FBC (Invitrogen, USA), 0.9 M dexamethasone (Sigma–Aldrich, USA) and 10 M insulin (Sigma–Aldrich, USA). Medium was replaced with serum- and hormone-free WME (Ikeda et al., 2007).

2.8. DNA fragmentation assay

The cell apoptosis was determined by DNA fragmentation assay. Briefly, isolated hepatocyte was lysed in 1% NP-40 buffer containing 20 mM EDTA, 50 mM Tris–HCl, pH 7.5 for 1 min with thorough