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Original Article

Hepatoprotective effect of the ethanol extract of *Polygonum orientale* on carbon tetrachloride-induced acute liver injury in mice

Q3 *Yung-Jia Chiu* [a], *Shen-Chieh Chou* [b], *Chuan-Sung Chiu* [c], *Chun-Pin Kao* [c], *Kun-Chang Wu* [a], *Chao-Jung Chen* [d,e], *Jen-Chieh Tsai* [f,*], *Wen-Huang Peng* [a,**]

[a] *Department of Chinese Pharmaceutical Sciences and Chinese Medicine Resources, College of Biopharmaceutical and Food Sciences, China Medical University, 91 Hsueh-Shih Road, Taichung, 40402, Taiwan, ROC*
[b] *School of Pharmacy, College of Pharmacy, China Medical University, 91 Hsueh-Shih Road, Taichung, 40402, Taiwan, ROC*
[c] *Hsin Sheng College of Medical Care and Management, 418, Sec. Gaoping, Zhongfeng Road, Longtan District, 32544, Taoyuan, Taiwan, ROC*
[d] *Proteomics Core Laboratory, Department of Medical Research, China Medical University Hospital, 91 Hsueh-Shih Road, Taichung, 40402, Taiwan, ROC*
[e] *Graduate Institute of Integrated Medicine, China Medical University, 91 Hsueh-Shih Road, Taichung, 40402, Taiwan, ROC*
[f] *Department of Medicinal Botanicals and Health Applications, College of Biotechnology and Bio-Resources, Da-Yeh University, 168 University Road, Dacun, Chang-Hua, 51591, Taiwan, ROC*

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ABSTRACT

Polygonum orientale L. (Polygonaceae) fruits have various medicinal uses, but their hepatoprotective effects have not yet been studied. This study investigated the hepatoprotective activity of the ethanolic extract of *P. orientale* (POE) fruits against carbon tetrachloride (CCl_4)-induced acute liver injury (ALI). Mice were pretreated with POE (0.1, 0.5, and 1.0 g/kg) or silymarin (0.2 g/kg) for 5 consecutive days and administered a dose of 0.175% CCl_4 (ip) on the 5th day to induce ALI. Blood and liver samples were collected to measure antioxidative activity and cytokines. The bioactive components of POE were identified through high-performance liquid chromatography (HPLC). Acute toxicity testing indicated that the LD_{50} of POE exceeded 10 g/kg in mice. Mice pretreated with POE (0.5, 1.0 g/kg) experienced a significant reduction in their serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), and alkaline phosphatase (ALP) levels and reduction in the extent of liver lesions. POE reduced the malondialdehyde (MDA), nitric oxide (NO), tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) levels, and increased the activity of superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GRd) in liver. HPLC revealed peaks at 11.28, 19.55, and 39.40 min for protocatechuic acid, taxifolin, and quercetin, respectively. In summary, the

* *Corresponding author*. Fax: +886 4 85811326.
** *Corresponding author*. Fax: +886 4 224075683.
E-mail addresses: jenchieh@mail.dyu.edu.tw (J.-C. Tsai), whpeng@mail.cmu.edu.tw (W.-H. Peng).

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hepatoprotective effect of POE against CCl_4-induced ALI was seemingly associated with its antioxidant and anti-proinflammatory activities.

1. Introduction

The liver is a crucial organ in the human body. It regulates functions including the synthesis of proteins, secretion of biochemical enzymes, and detoxification of xenobiotics. Acute liver injury (ALI) is usually caused by toxic chemicals, drugs, or pathogen infections [1]. Carbon tetrachloride (CCl_4), a well-known agent used in animal models of hepatotoxicity, is metabolized by cytochrome P450 enzymes into highly reactive trichloromethyl radicals that can activate Kupffer cells and mediate the hepatic inflammation process by producing proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) [2,3]. Lipid peroxidation is also caused by trichloromethyl radicals [4]. Necrotic or apoptotic cells are regenerated and replaced by parenchymal cells after ALI. This process is associated with an inflammatory response and a limited extracellular matrix (ECM) deposition. If the hepatic injury persists, liver regeneration eventually fails and hepatocytes are replaced by a large amount of ECM that induces fibrillar collagen. Because continuous liver injury causes fibrosis, a reduction in ALI might avert this outcome [2].

Polygonum orientale L. (Polygonaceae) has been used as medicine and food in Chinese herbal pharmacopeias [5]. Numerous natural products possess medicinal properties; for example, flavonoids are among the most well-known examples of powerful pharmaceutical ingredients found in plants. Phytochemical studies have shown abundant flavonoids in this plant such as taxifolin and quercetin. Phenolics such as gallic acid and protocatechuic acid were also identified [6]. The abilities of various extracts of *P. orientale* (POE) to scavenge free radicals were reported in an *in vitro* study [5]. The whole plant has been used in China for the treatment of various conditions such as arthritis, edema, dysentery, fractures, urticarial, and muscle injuries [7]. Pharmacological studies have indicated that all parts of *P. orientale* possess anti-inflammatory, antifibrosis, antioxidative, anticancer, immune-stimulating, swelling-subsiding, antimyocardial ischemic, and vasodilating activities, and that these properties are concentrated in its fruit [5,8]. However, the hepatoprotective effects of this plant remain unknown.

2. Methods and materials

2.1. Chemicals and methods

Silymarin, taxifolin hydrate (≥90%), and quercetin (≥98%) were purchased from Sigma–Aldrich Chemical Co. (USA). Protocatechuic acid (≥98%) was purchased from Tokyo Chemical Industry Co. Ltd. (Japan). CCl_4 was purchased from Merck Co. (Germany) and was dissolved in olive oil to form a 0.175% (v/v) solution. Silymarin was suspended in 1% carboxymethylcellulose (CMC). All other reagents used were of analytical grades. Serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), alkaline phosphatase (ALP), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GRd) assay kits were purchased from Randox Laboratory Ltd., and nitric oxide (NO) and malondialdehyde (MDA) assays were obtained from Cayman Chemical Co. (Ann Arbor, MI, USA). TNF-α, IL-1β, and IL-6 were obtained from eBioscience Inc. (San Diego, CA, USA).

2.2. Preparation of POE

The fruits of POE were collected in Toushe, Nantou County, Taiwan in July 2013. This plant was identified by Professor Chao-Lin Kuo, Chair of the Department of Chinese Pharmaceutical Sciences and Chinese Medicine Resources, China Medical University (CMU-CMR). A voucher specimen (CMU-CMR-PO-103001) was deposited at CMU-CMR for further reference. The crude extract preparation procedure was modified from that described in a previous study [9]. Whole fruits were dried in a circulating air oven, crushed, and ground into a coarse powder (1.8 kg) that was extracted by triple maceration with 70% ethanol. The filtered extracts were collected and concentrated under reduced pressure to produce 91.5 g of extract with a yield of 5.08% (w/w). The remaining solution was lyophilized before the final POE was obtained. The extract was stored at −20 °C before the experiment.

2.3. Analysis of POE by high-performance liquid chromatography

High-performance liquid chromatography (HPLC) profiles were established for the standards (protocatechuic acid, taxifolin hydrate, and quercetin) and the POE. The methodology followed that of a previous study with minor modifications [10]. The analysis was performed on an Ascentis C18 column (4.6 mm × 250 mm × 5 μm) purchased from Sigma–Aldrich by using a Shimadzu LC-20AT HPLC and SPD-20A UV–Vis detector. Optimum separation was achieved using gradient elution with 0.1% aqueous phosphoric acid (A) and methanol (B) programmed as time and percentage of B: 0 min (10%), 10 min (39%), 20 min (47%), 30 min (47%), 40 min (58%), 55 min (56%), 65 min (10%), and 75 min (10%). All standards and samples were passed through a 0.45 μm Minipore filter before injection into the column with a sample size of 20 μL. The flow rate was set at 1.0 mL/min and detected at 270 nm. The chromatograms of the standards and POE are shown in Fig. 1.

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