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Glechoma hederacea extracts attenuate cholestatic liver injury in a bile duct-ligated rat model

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ABSTRACT

Ethnopharmacological relevance: In traditional Chinese medicine, *Glechoma hederacea* is frequently prescribed to patients with cholelithiasis, dropsy, abscess, diabetes, inflammation, and jaundice. Polyphenolic compounds are main bioactive components of *Glechoma hederacea*.

Aim of the study: This study was aimed to investigate the hepatoprotective potential of hot water extract of *Glechoma hederacea* against cholestatic liver injury in rats.

Materials and methods: Cholestatic liver injury was produced by ligating common bile ducts in Sprague-Dawley rats. Saline and hot water extract of *Glechoma hederacea* were orally administrated using gastric gavages. Liver tissues and bloods were collected and subjected to evaluation using histological, molecular, and biochemical approaches.

Results: Using a rat model of cholestasis caused by bile duct ligation (BDL), daily oral administration of *Glechoma hederacea* hot water extracts showed protective effects against cholestatic liver injury, as evidenced by the improvement of serum biochemicals, ductular reaction, oxidative stress, inflammation, and fibrosis. *Glechoma hederacea* extracts alleviated BDL-induced transforming growth factor beta-1 (TGF-β1), connective tissue growth factor, and collagen expression, and the anti-fibrotic effects were accompanied by reductions in α-smooth muscle actin-positive matrix-producing cells and Smad2/3 activity. *Glechoma hederacea* extracts attenuated BDL-induced inflammatory cell infiltration/accumulation, NF-κB and AP-1 activation, and inflammatory cytokine production. Further studies demonstrated an inhibitory effect of *Glechoma hederacea* extracts on the axis of high mobility group box-1 (HMGB1)/toll-like receptor-4 (TLR4) intracellular signaling pathways.

Conclusions: The hepatoprotective, anti-oxidative, anti-inflammatory, and anti-fibrotic effects of *Glechoma hederacea* extracts seem to be multifactorial. The beneficial effects of daily *Glechoma hederacea* extracts supplementation were associated with anti-oxidative, anti-inflammatory, and anti-fibrotic potential, as well as down-regulation of NF-κB, AP-1, and TGF-β/Smad signaling, probably via interference with the HMGB1/TLR4 axis.

Abbreviations: ALT, alanine aminotransferase; ANOVA, one-way analysis of variance; AST, aspartate aminotransferase; BDL, bile duct ligation; CTGF, connective tissue growth factor; ELISA, enzyme-linked immunosorbent assay; EMSA, electrophoretic mobility shift assay; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; γ-GT, γ-glutamyl transpeptidase; H & E, hematoxylin/eosin; HMGB1, high mobility group box-1; HPLC, high performance liquid chromatography; IL-1β, interleukin-1β; MDA, malondialdehyde; MMP-2, matrix metalloproteinase-2; MMP-9, matrix metalloproteinase-9; MPO, Myeloperoxidase; ROS, reactive oxygen species; RT-PCR, reverse transcriptase polymerase chain reaction; α-SMA, α-smooth muscle actin; TAK1, transforming growth factor β-activated kinase-1; TBARS, thiobarbituric acid reactive substances; TGF-β1, transforming growth factor beta-1; TIMP-1, tissue inhibitor of metalloproteinase-1; TIMP-2, tissue inhibitor of metalloproteinase-2; TLR4, toll-like receptor-4; TNF-α, tumor necrosis factor-α

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1. Introduction

The liver orchestrates complicated metabolic networks involved in homeostatic body functions and its dysregulation usually causes acute or chronic liver diseases. Bile acids, which are critical detergent molecules and metabolic integrators, are dynamically regulated by hepatic synthesis and hepatobiliary secretion and reabsorption. The abnormal accumulation of bile acids caused by structural and functional impairment of hepatobiliary systems predisposes the liver to cholestasis (Li and Chiang, 2014). It has been shown that familial genetic disorders, autoimmune diseases, xenobiotic exposure, gallstones, and tumors are common causes of cholestasis (Li and Crawford, 2004). Together with an elevation and accumulation of bile acids and other toxins in liver and plasma, cholestasis is also accompanied by a series of biochemical changes, including cholangiocyte proliferation, ductular reaction, hepatocellular injury, inflammatory cell infiltration/activation, oxidative stress, myofibroblast proliferation/activation, fibrosis, and even cirrhosis (Kim et al., 2015; Li and Crawford, 2004; O'Brien et al., 2013). Malnutrition, muscle wasting, and impaired glucose/lipid metabolism are also signs of chronic cholestasis (Lin et al., 2005, 2016). Among the accumulated hepatic toxins, hydrophobic bile acids show apparent detrimental effects which induce hepatic inflammation, generate reactive oxygen species (ROS), and impact hepatocytes, bile duct epithelial cells, cholangiocytes, stellate cells, and Kupffer cells (Lamireau et al., 2003; Li and Chiang, 2014; O'Brien et al., 2013). Therefore, these findings highlight the crucial roles of bile acids and the aforementioned biochemical events in the pathogenesis of cholestasis, and suggest they could be potential candidates as therapeutic targets in treating cholestatic liver diseases.

Pharmaceutical plants, functional foods, vegetables, and fruits are rich in biologically active compounds and possess a wide spectrum of pharmacological properties capable of conferring health benefits. Thus, herbal medicines have been used in therapeutic and/or preventive medicine as alternatives to or to complement conventional drugs (Atefipour et al., 2016; Chen et al., 2015). *Glechoma hederacea* var. *longituba* Nakai, also known as gill-over-the-ground and creeping Charlie, is an aromatic creeper of the mint family Lamiaceae. In traditional oriental and Chinese medicine, *Glechoma hederacea* is frequently prescribed to patients with cholelithiasis, dropsy, abscess, diabetes, inflammation, and jaundice (Kumarasamy et al., 2002; Vogl et al., 2013). Supporting evidence indicates that *Glechoma hederacea* extracts possess several biological activities, including depigmentation, anti-melanogenesis, anti-osteoclastogenesis, anti-tumor, anti-xanthine oxidase, anti-oxidation, and anti-inflammation (An et al., 2006; Ha et al., 2011; Hwang et al., 2014; Kim et al., 2011; Masuda et al., 2013; Milovanovic et al., 2010; Ohigashi et al., 1986; Qiao et al., 2012). Though *Glechoma hederacea* has been extensively used in folk remedies, including traditional Chinese medicine, for relieving symptoms of inflammation, oxidation, and jaundice, there is currently no experimental evidence showing its effectiveness against liver diseases.

Common bile duct ligation (BDL) and scission in rodents is used as an experimental model of extrahepatic cholestasis. Obstructive cholestasis is initially indicated by portal hypertension and bile acid accumulation which progresses to liver injury, portal fibrosis, biliary cirrhosis, and even liver failure. During the progressive course, inflammation and oxidative stress play crucial pathological roles. Thus, anti-inflammatory and/or anti-oxidative treatments are beneficial in BDL-induced cholestatic liver injury (Atefipour et al., 2016; Chen et al., 2012; Lin et al., 2014; Pan et al., 2014). In traditional folk medicine, *Glechoma hederacea* is prepared as a beverage by boiling with hot water. It has been reported that hot water extract of *Glechoma hederacea* possesses antioxidant activity with potency greater than that of vitamin C and Trolox (Chou et al., 2012). The use of *Glechoma hederacea* in traditional remedies and its ameliorative effects on inflammation and oxidative stress motivated the authors of the present study to consider the feasibility of applying it to treat cholestasis. To extend its clinical relevance, we aimed to ascertain the hepatoprotective potential of hot water extract of *Glechoma hederacea* using a BDL-induced cholestasis rat model and examined its effects on hepatic inflammation, oxidative stress, and fibrosis with a view to identifying the underlying molecular basis.

2. Material and methods

2.1. Preparation of Glechoma hederacea extracts

Naturally growned *Glechoma hederacea* was obtained from Taichung City, Taiwan. Voucher specimen were identified by Dr. Bing-Shiunn Chen and deposited in Department of Horticulture, National Chung Hsing University, Taichung City, Taiwan (No. NCHU-2016-001). The *Glechoma hederacea* extracts were prepared according to our previously reported procedures (Chou et al., 2012). Briefly, the whole plants of *Glechoma hederacea* were cut into small pieces and extracted using 1:50 (w/v) of plants and distilled water (100 °C for 3 h, hot water extracts). The decoctions were filtered, lyophilized, and stored at −70 °C until use.

2.2. High performance liquid chromatography (HPLC) analysis

To determine the contents of polyphenolic compounds in *Glechoma hederacea* extracts, HPLC analysis (Hewlett-Packard HPLC System, HP 1100 series, Waldron, Germany) was performed according to our previously described method with modification (Kao et al., 2010). After filtration through a 0.45 μm filter, the extracts were injected into the column and mobilized with a stepwise gradient of water (0.1% v/v phosphoric acid)-acetonitrile (0.01 min, 94:6; 50 min, 65:35). The nature of the eluted compounds was identified by matching the retention time and spectrum with those of known standards.

2.3. Animals and BDL operation

Adult male Sprague-Dawley rats (200–250 g) were purchased from BioLASCO (Taipei, Taiwan) and housed in a controlled animal facility at a constant temperature, with a daily 12 h light dark cycle, and free access to regular chow and water *ad libitum*. All experimental procedures involving animals were carried out with efforts to minimize suffering in accordance with the policies of the Institutional Animal Care and Use Committee and with the approval of Taichung Veterans General Hospital. The rats were randomly allocated to five experimental groups (n =8 per group). After anesthesia with isoflurane, BDL in rats was produced by ligating the common bile duct, and an abdominal incision without a ligation served as the sham operation (Pan et al., 2014). In the sham group, rats were fed with saline vehicle or 2 g/kg of hot water extract of *Glechoma hederacea*. BDL rats received saline vehicle, 0.5, or 2 g/kg of *Glechoma hederacea*. Saline and hot water extract of *Glechoma hederacea* were orally administrated using gastric gavages once daily at 9–10 AM for 4 weeks starting 1 week before surgical operation. At the end of 3 weeks after surgery, all rats were anesthetized with isoflurane and sacrificed for further analyses. We had previously reported that daily administration of *Graptopetalum paraguayense* E. Walther leaf extracts at a dose of 2 g/kg protected against brain injury in rats (Kao et al., 2010). Thus, the choice of treating doses was made according to our relevant study with some modifications.

2.4. Biochemical analysis

Levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and γ-glutamyl transpeptidase (γ-GT), were measured by automated standardized procedures (Roche Hitachi 917/747, Mannheim, Germany). The concentrations of total serum bile acids were measured using a total bile acids assay kit (Diazyme Laboratories,