Isorhamnetin-3-\textit{O}-galactoside Protects against CCl\textsubscript{4}-Induced Hepatic Injury in Mice

Dong-Wook Kim\textsuperscript{1}, Hong-Ik Cho\textsuperscript{1}, Kang-Min Kim\textsuperscript{1}, So-Jin Kim\textsuperscript{1}, Jae Sue Choi\textsuperscript{2}, Yeong Shik Kim\textsuperscript{3} and Sun-Mee Lee\textsuperscript{1,*}

\textsuperscript{1}School of Pharmacy, Sungkyunkwan University, Suwon 440-746,  
\textsuperscript{2}Faculty of Food Science and Biotechnology, Pukyong National University, Busan 608-737,  
\textsuperscript{3}College of Pharmacy, Seoul National University, Seoul 151-742, Republic of Korea

Abstract

This study was performed to examine the hepatoprotective effect of isorhamnetin-3-\textit{O}-galactoside, a flavonoid glycoside isolated from \textit{Artemisia capillaris} Thunberg (Compositae), against carbon tetrachloride (CCl\textsubscript{4})-induced hepatic injury. Mice were treated intraperitoneally with vehicle or isorhamnetin-3-\textit{O}-galactoside (50, 100, and 200 mg/kg) 30 min before and 2 h after CCl\textsubscript{4} (20 \textmu g/kg) injection. Serum aminotransferase activities and hepatic level of malondialdehyde were significantly higher after CCl\textsubscript{4} treatment, and these increases were attenuated by isorhamnetin-3-\textit{O}-galactoside. CCl\textsubscript{4} markedly increased serum tumor necrosis factor-\alpha level, which was reduced by isorhamnetin-3-\textit{O}-galactoside. The levels of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and heme oxygenase-1 (HO-1) protein and their mRNA expression levels were significantly increased after CCl\textsubscript{4} injection. The levels of HO-1 protein and mRNA expression levels were augmented by isorhamnetin-3-\textit{O}-galactoside. The levels of phosphorylated c-Jun N-terminal kinase, extracellular signal-regulated kinase and p38, and isorhamnetin-3-\textit{O}-galactoside reduced these increases. The nuclear translocation of nuclear factor kappa B (NF-\kappa B) and c-Jun nuclear translocation, while it augmented the nuclear level of Nrf2. These results suggest that isorhamnetin-3-\textit{O}-galactoside ameliorates CCl\textsubscript{4}-induced hepatic damage by enhancing the anti-oxidative defense system and reducing the inflammatory signaling pathways.

Key Words: Carbon tetrachloride, Heme oxygenase-1, Hepatotoxicity, Inflammation, Isorhamnetin-3-\textit{O}-galactoside, Oxidative stress

INTRODUCTION

Acute and chronic liver diseases constitute a global concern and the concern is worsened by the lack of reliable liver protective drugs, despite the increasing need for agents to protect the liver from damage. Therefore, complementary and alternative medicines for the treatment of liver diseases have been receiving considerable interest (Seeff et al., 2001). Therapeutically effective agents from natural products may reduce the risk of clinical toxicity.

Carbon tetrachloride (CCl\textsubscript{4}) has long been known as a toxicant and has been widely used in many \textit{in vitro} and \textit{in vivo} toxicology studies. CCl\textsubscript{4} causes liver toxicity, resulting in cellular necrosis, fatty degeneration, fibrosis and cirrhosis (Talib et al., 2005). Administered CCl\textsubscript{4} is metabolized by cytochrome P450 (CYP), primarily CYP2E1 and results in the formation of trichloromethyl radical (·CCl\textsubscript{3}), which initiates lipid peroxidation and protein oxidation leading to hepatocellular damage (Manibusan et al., 2007).

\textit{Artemisia capillaris} Thunb. (Compositae) is one of the oldest and most commonly prescribed herbs in Eastern traditional medicine, and has been used as an analgesic, antimicrobial agent and a remedy for the treatment of hepatitis and bilious disorders (Chang and But, 1987). An aqueous extract of \textit{Artemisia capillaris} was shown to inhibit interleukin (IL)-1 receptor- and tumor necrosis factor (TNF) receptor-induced cytotoxicity and ethanol-induced apoptosis of HepG2 cells (Koo et al., 2002). A previous study reported that \textit{Artemisia capillaris} reduced the lipopolysaccharide-induced inflammatory response in a human hepatoma cell line and in the rat liver (Hong et al., 2004), and prevented 2,2'-azobis(2-amidino-propane) dihydrochloride-induced liver damage in rats (Han et
Isorhamnetin-3-O-galactoside (Fig. 1), one of the flavonoid constituents from Artemisia capillaris, exerted anti-inflammatory activity by inhibiting the production of 5-lipoxygenase-induced leukotriene (Kwon et al., 2011).

Therefore, this study was designed to investigate the protective effects of isorhamnetin-3-O-galactoside against CCl₄-induced acute hepatic injury, with particular attention to the oxidative stress and inflammatory pathways.

**MATERIALS AND METHODS**

**Isolation of isorhamnetin-3-O-galactoside from an Artemisia capillaris**

The whole plant of Artemisia capillaris Thunb. was dried and grinded to powder. The dried powder (9.5 kg) was then extracted with hot MeOH (50.0 l×3 times) for 3 h. After filtration, total filtrate was concentrated to dryness in vacuo at 40°C to obtain the MeOH extract (900 g). Following this, the MeOH extract was suspended in distilled water:MeOH (9:1) and MeOH fractions were chromatographed in a silica gel column using CH₂Cl₂:MeOH (6.8 g), respectively. 60% MeOH subfraction was decanted of subfraction 6F-3 yielding isorhamnetin-3-O-galactoside (270 mg).

**Histological analysis**

Liver tissues were removed from a portion of the left lobe, and fixed immediately in 10% neutral buffered formalin, embedded in paraffin, and then sectioned at 5 μm thickness. Serial sections were stained with hematoxylin and eosin for evaluation of portal inflammation, hepatocellular necrosis, and inflammatory cell infiltration. The sections were examined in a blind manner under an Olympus CKX 41 microscope (Olympus optical Co. Ltd., Tokyo, Japan).

**Lipid peroxidation**

The steady-state level of malondialdehyde (MDA), which is the end product of lipid peroxidation, was analyzed in liver homogenates by spectrophotometric measurement of the levels of thiobarbituric acid reactive substances at 535 nm, as described by Buege and Aust (1978), using 1,1,3,3-tetraethoxypropane (Sigma, St. Louis, MO, USA) as the standard.

**Serum TNF-α level**

Serum concentration of TNF-α was quantified using a commercial TNF-α enzyme-linked immunosorbent assay (ELISA) kit (BD Biosciences Co., CA, USA).

**Western blot immunoassay**

Freshly isolated liver tissue was homogenized in lysis buffer for the preparation of whole protein extracts. NE-PER® (Pierce Biotechnology, Rockford, IL, USA) was used for the extraction of nuclear proteins according to the manufacturer’s instructions. The BCA Protein Assay kit (Pierce Biotechnology) was used to determine protein concentrations. Protein samples were loaded on 10-15% polyacrylamide gels, separated by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS/PAGE) and then transferred to PVDF membranes (Millipore, Billerica, MA, USA) using the Semi-Dry Trans-Blot Cell (Bio-Rad Laboratories, Hercules, CA, USA). After the transfer, the membranes were washed with 0.1% Tween-20 in 1×Tris Buffered Saline (TBS/T) and blocked for 1 h at room temperature with 5% (w/v) skim milk powder in TBS/T. The blots were then incubated overnight at 4°C with primary antibodies. The