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Aqueous extract of *Artemisia iwayomogi* Kitamura attenuates cholestatic liver fibrosis in a rat model of bile duct ligation

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ABSTRACT

Cholestatic liver fibrosis, characterized by excessive accumulation of extracellular matrix (ECM) proteins, is associated with bile acid-induced oxidative stress and lipid peroxidation. We evaluated the therapeutic or protective effect of an aqueous extract of *Artemisia iwayomogi* Kitamura (WAI) in a rat bile duct ligation (BDL)-induced hepatic fibrogenesis model. After BDL, rats were treated once daily with 25 or 50 mg/kg of WAI for 2 weeks. The serum bilirubin, aspartate transaminase, alanine transaminase, malondialdehyde, and liver hydroxyproline levels were drastically increased in the BDL group. WAI administration significantly reduced these markers and restored BDL-induced depletion of glutathione content and glutathione peroxidase activity. Cholestatic liver injury and collagen deposition were markedly attenuated by WAI treatment, and these changes were paralleled by significantly suppressed gene and protein expression of fibrogenic factors, including hepatic alphasmooth muscle actin, platelet-derived growth factor, and transforming growth factor β. Our data suggest that WAI may have antifibrotic properties via both improvement of antioxidant activities and inhibition of ECM protein production in the rat model of BDL.

1. Introduction

Liver fibrosis is characterized by excessive accumulation of extracellular matrix (ECM) proteins such as type I and type IV collagen within the perisinusoidal space of Disse (Dai et al., 2009). It is a major feature of most chronic liver injuries, including metabolic, viral, cholestatic, and biliary disorders (Haber et al., 2008; Henderson and Forbes, 2008; Pinzani and Rombouts, 2004). Abnormal flux of bile acids and bilirubin in the liver are hallmarks of cholestasis, which leads to retention and accumulation of toxic hydrophobic bile salts within hepatocytes (Faubion et al., 1999), causing inflammatory reactions, hepatocyte death, and periductular fibrosis (Webster and Anwer, 1998).

Mechanisms of cholestatic liver damage are complex, and bile salt-mediated fibrosis is not completely understood. Nevertheless, direct or indirect toxic effects of retained bile acids as well as inflammation and excessive oxidative stress are known as important factors (Chen et al., 2009; Dold et al., 2009). Results from bile duct ligation (BDL) animal model studies suggest that these factors should be considered as potential targets for therapeutic intervention in cholestatic liver injury (Liu et al., 2001; Ljubuncic et al., 2000; Song et al., 2008). In particular, oxidative stress is likely to play a key role in cholestasis-induced liver fibrosis, as evidenced by reduced liver injury and fibrosis after BDL in mice lacking NADPH oxidase (NOX-1), which produces reactive oxygen species (ROS) (Cui et al., 2011).

For thousands of years, herbal medicines have been widely used as hepatoprotective and antifibrotic drugs in the treatment of liver diseases (Dhiman and Chawla, 2005; Lee et al., 2007a,b; Lin et al., 2011). *Artemisia iwayomogi* Kitamura is a medicinal plant with hepatotherapeutic effects and is used in traditional medicine in Korea and China (Jang, 1975; Zhang, 1978). The pharmacological activities of *A. iwayomogi* and its major compound, scopoletin, have been previously shown to have anti-inflammatory (Shin et al., 2006), antimicrobial (Seo et al., 2010), antioxidant (Kim et al., 2004; Seo and Yun, 2008), and hepatoprotective effects (Choi et al., 2005; Wang et al., 2012). Two studies have proposed that *A. iwayomogi* has antifibrotic effects in carbon tetrachloride (CCl_4) injury rat models (Park et al., 2000; Wang et al., 2012).

To date, no studies have examined whether *A. iwayomogi* is effective in treating pathological conditions associated with

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cholestatic liver injury. With an increasing number of patients exhibiting metabolic disorders, hepatic cholestasis and cholestatic liver fibrosis have become important medical issues (Aller et al., 2008).

In the present study, we evaluated the potential hepatoprotective and antifibrotic effects of an aqueous extract of *A. iwayomogi* (WAI) in a cholestatic liver fibrosis animal model.

2. Materials and methods

2.1. Reagents and chemicals

Hydroxyproline, 6,7-dimethoxycoumarin (scoparone), 7-hydroxy-6-methoxycoumarin (scopoletin), p-dimethylaminobenzaldehyde, 1,1,3,3-tetraethoxypropane (TEP), chloramines-T, 5,5-dithiobis-2-nitrobenzoic acid (DTNB), reduced glutathione, glutathione reductase (GSH-rd), glutathione peroxidase (GSH-px), β-nicotinamide adenine dinucleotide phosphate (β-NADP) , and β-NADPH were purchased from Sigma–Aldrich (St. Louis, MO). Perchloric acid was obtained from GFS Chemical Co. (Columbus, OH); thiobarbituric acid (TBA), from Lancaster Co. (Lancashire, England, UK); and hydrogen peroxide, from Junsei Chemical Co., Ltd. (Tokyo, Japan). Anti-α-smooth muscle actin mouse monoclonal antibody was purchased from Abcam (Cambridge, UK); Histofine, from Nichirei Biosciences (Tokyo, Japan); diaminobenzidine (DAB), from Abcam; and Mayer's hematoxylin, from Wako Pure Chemical Industries (Osaka, Japan).

2.2. Preparation of WAI and HPLC-based fingerprinting

An aqueous extract of *A. iwayomogi* (WAI) was prepared as described previously (Wang et al., 2012). Briefly, *A. iwayomogi* was washed twice with distilled water and fully dried in an oven at 60 °C. Then, 100 g of *A. iwayomogi* were boiled in 1 L of distilled water for 30 min and concentrated at 60 °C for 120 min. After filtration and lyophilization, the final yield was 4.33% (w/w). The dried extract was dissolved in distilled water before use; the remainder was stored at −70 °C for future use.

For verification, a fingerprint analysis of *A. iwayomogi* was conducted by two-dimensional high-performance liquid chromatography (HPLC), using scopoletin and scoparone as positive and negative references, respectively. Briefly, 5 mg of WAI or 0.1 mg each of scopoletin and scoparone were dissolved in 1 ml of 50% methanol, and the samples were filtered and analyzed by HPLC. The HPLC system consisted of a Thermo Accela Ultra Performance LC (UPLC) system, an Acquity UPLC binary solvent manager, an Acquity sample manager/column heater module, an Acquity photo-diode array detector system, and an Acquity UPLC BEH C18 column (1.7 μm; 2.1 × 100 mm) (Waters, Milford, MA). The compounds were eluted at a flow rate of 0.3 ml/min with a gradient from 95% solvent A (0.1% formic acid in water) and 5% solvent B (0.1% formic acid in acetonitrile) to 0% A and 100% B over 20 min, followed by a return to 95% A and 5% B over 25 min. All chromatograms were obtained by detection at a wavelength of 345 nm (Fig. 1).

2.3. Animals

Specific pathogen-free 6-week-old male Sprague–Dawley rats were purchased from a commercial animal breeder (Koatech, Gyeonggi Do, Korea). Forty rats were acclimated in an environmentally controlled room at 22 ± 2 °C, 55% ± 10% relative

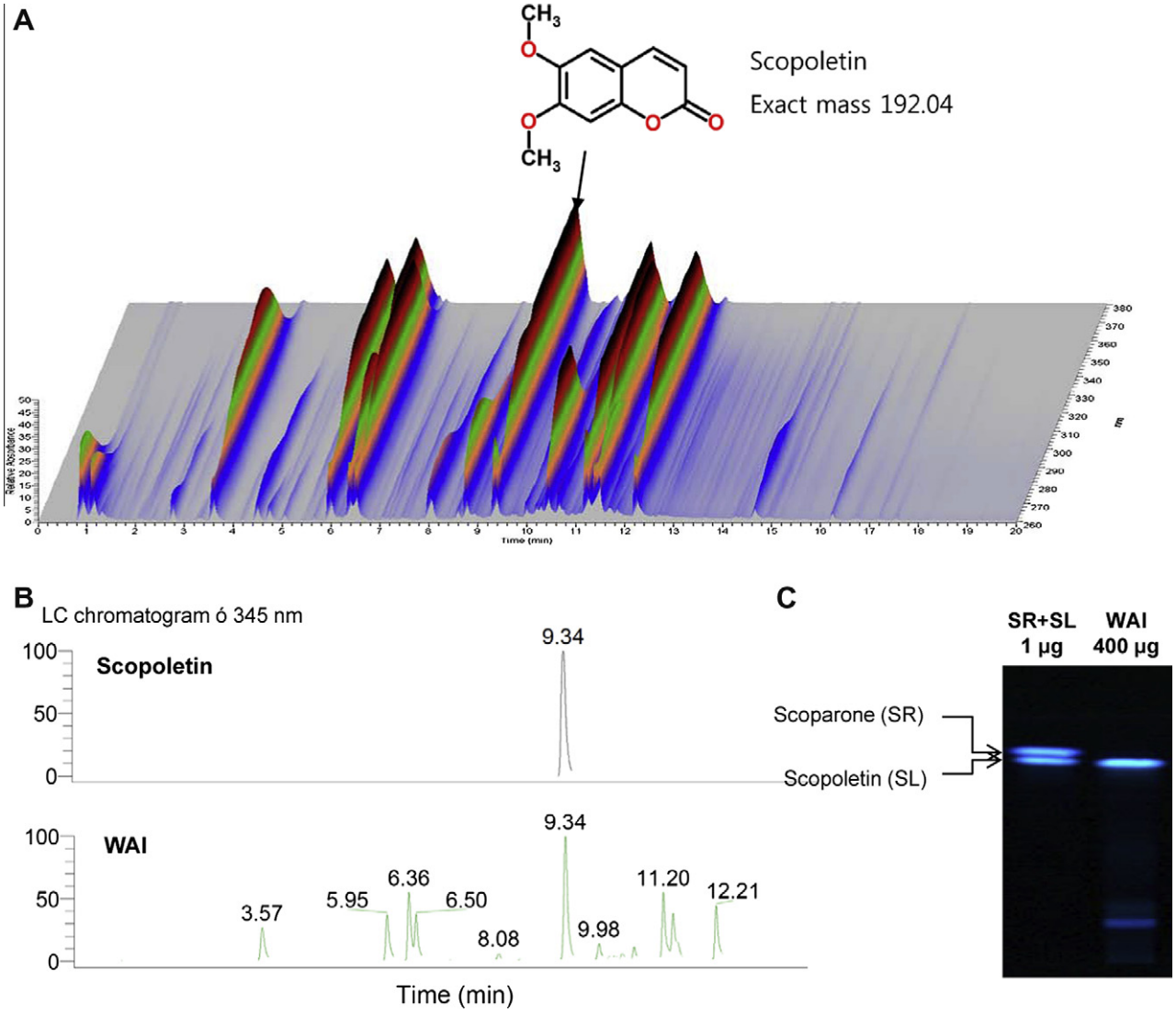

Fig. 1. HPTLC- and HPLC-based fingerprint analyses of WAI. Fingerprint analysis was performed to characterize WAI and its major components, using 7-hydroxy-6-methoxycoumarin (scopoletin) as a reference compound. A three-dimensional chromatogram (A) and fingerprints of major active compounds (B) were determined by HPLC–DAD–MS. HPTLC-based fingerprints of positive and negative controls were also obtained (C).