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Original Article

The essential oil of *Artemisia capillaris* protects against CCl_4-induced liver injury *in vivo*

Qinghan Gao[a,b,1], Xin Zhao[a,1], Lei Yin[a,1], Yuanbin Zhang[a], Bing Wang[a], Xiuli Wu[a], Xinhui Zhang[a], Xueyan Fu[a,c,*], Weihong Sun[d,*]

[a] *School of Pharmacy, Ningxia Medical University, Yinchuan, China*
[b] *School of Public Health, Ningxia Medical University, Yinchuan, China*
[c] *Key Laboratory of Hui Ethnic Medicine Modernization, Ministry of Education (Ningxia Medical University), Yinchuan, China*
[d] *General Hospital of Ningxia Medical University, Yinchuan, China*

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ABSTRACT

To study the hepatoprotective effect of the essential oil of *Artemisia capillaris* Thunb., Asteraceae, on CCl_4-induced liver injury in mice, the levels of serum aspartate aminotransferase and alanine aminotransferase, hepatic levels of reduced glutathione, activity of glutathione peroxidase, and the activities of superoxide dismutase and malondialdehyde were assayed. Administration of the essential oil of *A. capillaris* at 100 and 50 mg/kg to mice prior to CCl_4 injection was shown to confer stronger *in vivo* protective effects and could observably antagonize the CCl_4-induced increase in the serum alanine aminotransferase and aspartate aminotransferase activities and malondialdehyde levels as well as prevent CCl_4-induced decrease in the antioxidant superoxide dismutase activity, glutathione level and glutathione peroxidase activity ($p < 0.01$). The oil mainly contained β-citronellol, 1,8-cineole, camphor, linalool, α-pinene, β-pinene, thymol and myrcene. This finding demonstrates that the essential oil of *A. capillaris* can protect hepatic function against CCl_4-induced liver injury in mice.

Introduction

Liver disease, a common disorder caused by viral hepatitis, alcoholism, liver-toxic chemicals, unhealthy dietary habits and environmental pollution, is a global concern (Papay et al., 2009). However, medical treatment for this disease is often hard to administer and has a confined effect. Traditional Chinese herbal medicines, which underlie numerous prescriptions used to treat liver diseases, are still widely used by the Chinese (Zhao et al., 2014). *Artemisia capillaris* Thunb., Asteraceae, according to the Bencao Gangmu, the most famous records of Chinese Traditional Medicine, has been widely used as a medicine to clear heat, promote diuresis and remove jaundice and has also been used as a flavor in beverages, vegetables, and pastries because of its particular fragrance. *A. capillaris* has been regarded as a type of Chinese folk medicine and food by a growing number of people. Therefore, there have been considerable efforts to develop useful herbal medicines, such as *A. capillaris*, for the treatment of liver disease.

In recent years, herbal medicines have gained more attention and popularity for the treatment of liver disease because of their safety and efficacy (Ding et al., 2012). *A. capillaris* has been proven to possess good hepatoprotective activity based on modern pharmacological methods (Han et al., 2006). It is also an important medicinal material in China and is a popular anti-inflammatory (Cha et al., 2009a), choleretic (Yoon and Kim, 2011), and anti-tumor (Feng et al., 2013) herbal remedy.

Phytochemical studies have revealed a number of volatile essential oils, coumarins, and flavonol glycosides as well as a group of unidentified aglycones from *A. capillaris* (Komiya et al., 1976; Yamahara et al., 1989). The essential oil of *A. capillaris* (AEO) is one of the main pharmacological active compounds and confers anti-inflammatory (Cha et al., 2009a) and anti-apoptotic properties (Cha et al., 2009b). However, as AEO is one of the main compounds of *A. capillaris*, the potential hepatoprotective activities of the major constituents from *A. capillaris* should be explored.

In this study, the protective effect of AEO on carbon tetrachloride (CCl_4)-induced hepatotoxicity was evaluated by biochemical methods, such as hepatic reduced glutathione (GSH),

* Corresponding authors.
E-mails: xueyanfu2661@163.com (X. Fu), tungbuo@163.com (W. Sun).
[1] These authors equally contributed to this manuscript; they should be considered first author.

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malondialdehyde (MDA) levels, superoxide dismutase (SOD), and glutathione peroxidase (GSH-P*x*) activity, as well as the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the serum. The extent of CCl_4-induced liver injury was also analyzed through histopathological observations, accompanied with phytochemical analysis by GC–MS to identify the constituents of AEO.

Materials and methods

Drugs and chemicals

The essential oil of *Artemisia capillaris* Thunb., Asteraceae, was obtained from Kangshen Natural oils Co., Ltd (Jishui, Jiangxi Province, China). CCl_4 was purchased form Damao Chemical Company (Tianjin, China). Bifendate tablets were purchased from Yunpeng, Shanxi Pharmaceutical Co., Ltd. (no. A130602). The diagnostic kits used for the determination of ALT, AST, SOD, MDA, GSH and GSH-P*x* were obtained from the Nanjing Jiancheng Institute of Biotechnology (Nanjing, China).

AEO analysis

AEO was analyzed by gas chromatography–mass spectrometry (GC/MS) using a Shimadzu QP2010 plus GC with Rxi-5Sil MS (30 m × 0.25 mm; 0.25 μm film thickness). The GC/MS was run under the following conditions: a fused-silica capillary column with helium at 1.60 ml/min and an injector at 250 °C. The GC oven temperature was initially held at 40 °C for 5 min. Thereafter, the temperature was raised with a gradient of 3 °C min^{-1} until it reached 230 °C. This temperature was held for 3 min. Finally, the temperature was then raised with a gradient of 1 °C min^{-1} until 260 °C and again held for 10 min. Mass spectra were taken at 70 eV from 33 to 500 Da. Identification of the compounds was based on the comparison of the mass spectral data on a computer matched with NIST (similarity index >85%) and those described in the literature (Sylvestre et al., 2006; Cheng et al., 2008; Ait-Ouazzou et al., 2012; Argyropoulou and Skaltsa, 2012; Ćavar et al., 2012; Ben Mansour et al., 2013; Chen et al., 2013; Gouveia and Castilho, 2013; Murugan and Mallavarapu, 2013; Singh et al., 2013; Bagheri et al., 2014; Da Silva et al., 2014; Desai et al., 2014; Pandey et al., 2014; Qi et al., 2014; Rather et al., 2014; Sadgrove et al., 2014; Sereshti et al., 2014; Tao et al., 2014; Tian et al., 2014). The identification of compounds was performed according to their retention indices relative to C_8–C_{24} *n*-alkanes and MS.

Animals

Male ICR mice at 6–8 weeks of age (18–22 g) were purchased from the Medicine Experiment Animal Center of Ningxia Medicine University. The animals were allowed to acclimatize for two days to animal room conditions and were maintained on a standard pellet diet and water *ad libitum*. The food was withdrawn on the day before the experiment, but the animals were allowed free access to water. All animals were cared for in compliance with the Guide for the Care and Use of Laboratory Animals (1996). The experimental procedures were approved by our institutional animal research ethics committee (approval number: SYXK (NING) 2011-0001).

Rodent model design

The animals were randomly divided into five groups of fifteen mice each. The control group and model group mice were gavaged with sesame oil for 6 days. Positive control group mice were gavaged with bifendate tablets (BT, 10 mg/kg) for 6 days. The experimental groups were treated with 100 mg/kg and 50 mg/kg AEO dissolved in sesame oil for 6 days. On day 6, the control group was treated with sesame oil, and all of the other groups were treated with a single dose of 0.2% CCl_4 in sesame oil (10 ml/kg) by intraperitoneal injection. The mice were then fasted free of water, and blood samples were collected from the retrobulbar vessels; collected blood was centrifuged at 3000 × *g* for 10 min to separate the serum. Cervical dislocation was performed immediately after withdrawal of blood, and liver samples were promptly removed. One part of the liver sample was immediately stored at −20 °C until analysis, and another part was excised and fixed in a 10% formalin solution; the remaining tissues were stored at −80 °C for histopathological analysis (Wang et al., 2008; Hsu et al., 2009; Nie et al., 2015).

Measurement of the biochemical parameters in the serum

Liver injury was assessed by estimating the enzymatic activities of serum ALT and AST using the corresponding commercial kits according to the instructions for the kits (Nanjing, Jiangsu Province, China). The enzymatic activities were expressed as units per liter (U/l).

Measurement of MDA, SOD, GSH and GSH-Px in liver homogenates

Liver tissues were homogenized with cold physiological saline at a 1:9 ratio (w/v, liver:saline). The homogenates were centrifuged (2500 × *g* for 10 min) to collect the supernatants for the subsequent determinations. Liver damage was assessed according to the hepatic measurements of the MDA and GSH levels as well as the SOD and GSH-P*x* activities. All of these were determined following the instructions on the kit (Nanjing, Jiangsu Province, China). The results for MDA and GSH were expressed as nmol per mg protein (nmol/mg prot), and the activities of SOD and GSH-P*x* were expressed as U per mg protein (U/mg prot).

Histopathological analysis

Portions of freshly obtained liver were fixed in a 10% buffered paraformaldehyde phosphate solution. The sample was then embedded in paraffin, sliced into 3–5 μm sections, stained with hematoxylin and eosin (H&E) according to a standard procedure, and finally analyzed by light microscopy (Tian et al., 2012).

Statistical analysis

The results were expressed as the mean ± standard deviation (SD). The results were analyzed using the statistical program SPSS Statistics, version 19.0. The data were subjected to an analysis of variance (ANOVA, $p < 0.05$) followed by Dunnett's test and Dunnett's T_3 test to determine the statistically significant differences between the values of various experimental groups. A significant difference was considered at a level of $p < 0.05$.

Results and discussion

Constituents of AEO

Upon GC/MS analysis, the AEO was found to contain 25 constituents eluted from 10 to 35 min, and 21 constituents accounting for 84% of the essential oil were identified (Table 1). The volatile oil contained monoterpenoids (80.9%), sesquiterpenoids (9.5%), saturated unbranched hydrocarbons (4.86%) and miscellaneous acetylene (4.86%). Compared with other studies (Guo et al., 2004), we found abundant monoterpenoids (80.90%) in the AEO. The results showed that the most abundant constituent of AEO is β-citronellol (16.23%). Other major components of AEO include 1,8-cineole (13.9%), camphor (12.59%), linalool (11.33%), α-pinene