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In vivo hepatoprotective activity of the aqueous extract of *Artemisia absinthium* L. against chemically and immunologically induced liver injuries in mice

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ABSTRACT

Aim of the study: This study aimed to evaluate *in vivo* hepatoprotective activity of the aqueous extract of *Artemisia absinthium* L. (AEAA), which has been used for the treatment of liver disorders in Traditional Uighur Medicine.

Materials and methods: Qualitative and quantitative phytochemical analysis of the AEAA was performed by means of thin layer chromatography and spectrophometric assays. Aqueous extract (50, 100 or 200 mg/kg body weight/day) was administered orally to experimental mice. Liver injury was induced chemically, by a single CCl_4 administration (0.1% in olive oil, 10 ml/kg, i.v.), or immunologically, by injection of endotoxin (LPS, 10 μg, i.v.) in BCG-primed mice. The levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) in mouse sera, as well as superoxide dismutase (SOD), glutathione peroxidase (GPx) and malondialdehyde (MDA) in mouse liver tissues were measured. The biochemical observations were supplemented by histopathological examination.

Results: Obtained results demonstrated that the pretreatment with AEAA significantly ($P<0.001$) and dose-dependently prevented chemically or immunologically induced increase in serum levels of hepatic enzymes. Furthermore, AEAA significantly ($P<0.05$) reduced the lipid peroxidation in the liver tissue and restored activities of defense antioxidant enzymes SOD and GPx towards normal levels. In the BCG/LPS model, increase of the levels of important pro-inflammatory mediators TNF-α and IL-1 was significantly ($P<0.01$) suppressed by AEAA pretreatment. Histopathology of the liver tissue showed that AEAA attenuated the hepatocellular necrosis and led to reduction of inflammatory cells infiltration. Phytochemical analyses revealed the presence of sesquiterpene lactones, flavonoids, phenolic acids and tannins in the AEAA.

Conclusions: The results of this study strongly indicate the protective efect of AEAA against acute liver injury which may be attributed to its antioxidative and/or immunomodulatory activity, and thereby scientifically support its traditional use.

1. Introduction

Liver diseases are one of the most serious health problems in the world today but, despite tremendous advances in modern medicine, their prevention and treatment options still remain limited. However, the pathogenesis of hepatic diseases as well as the role of oxidative stress and inflammation therein is well established (Malhi and Gores, 2008; Tacke et al., 2009), and, accordingly, blocking or retarding the chain reactions of oxidation and inflammation process could be a promising therapeutic strategy for prevention and treatment of liver injury. Recently, the most common *in vivo* model used in the investigation of new hepatoprotective agents has been a well-characterized rodent model of liver injury induced by carbon tetrachloride (CCl_4), a chemical hepatotoxin that causes a free radical-mediated hepatocellular damage (Weber et al., 2003; Upur et al., 2009). Additionally, due to the numerous studies which revealed the important immune cell-mediated mechanisms involved in hepatocyte cell death, immunological liver injury induced by injection of endotoxin, bacterial cell wall lipopolysaccharide (LPS) into animals primed

Abbreviations: AEAA, aqueous extracts of *Artemisia absinthium*; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BCG, Bacillus Calmette–Guerin; CCl_4, carbon tetrachloride; CMC, sodium carboxymethylcellulose; GPx, glutathione peroxidase; IL-1, interleukin-1; LPS, lipopolysaccharide; MDA, malondialdehyde; ROS, reactive oxygen species; SOD, superoxide dismutase; TNF-α, tumor necrosis factor-α.

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with Bacillus Calmette–Guerin (BCG) constitutes another useful experimental model for evaluation of the antihepatotoxic efficacy (Zou et al., 2006; Corazza et al., 2009).

Effectively, herbal products are widely used in the treatment of hepatic disorders all over the world (Chaterrjee, 2000). An example of these can be found in Xinjiang Uighur Autonomous Region (North West of China) where medicinal plants have long been used in Traditional Uighur Medicine. *Artemisia absinthium* L. is a wild growing plant in this region and its aerial parts are used in Traditional Uighur Medicine, besides other conditions, for prevention and treatment of liver diseases (Chinese Pharmacopoeia, 2005). Previous phytochemical studies revealed that *A. absinthium* contains essential oil, bitter sesquiterpenoid lactones, flavonoids, phenolic acids and lignans (Wichtl, 1994). Several flavonol-3-glycosides have been isolated from leaves of *A. absinthium* and identified as quercetin, isorhamnetin, patuletin and spinacetin derivates (Hoffmann and Herrmann, 1982). *Artemisia absinthium* extracts have demonstrated to possess a strong antiradical and antioxidant activity *in vitro* (Canadanovic-Brunet et al., 2005), as well as antiparasitic activity in animal model (Caner et al., 2008). Although the aqueous-methanolic extract of *A. absinthium* has been reported to protect the liver against chemical toxins (Gilani and Janbaz, 1995), an extensive validation of hepatoprotective activity as well as elucidation of the underlying mechanisms was needed. Therefore, our work aimed to investigate protective effect of aqueous extract of *A. absinthium* (AEAA) against both, chemical and immunological liver injuries in animal model, in order to evaluate the claimed ethnopharmacological effect as well as to suggest the possible mechanisms of activity.

2. Materials and methods

2.1. Chemicals

Mycobacterium bovis Bacillus Calmette–Guerin (BCG) was purchased from the Institute of Lanzhou Biological Products (China), while lipopolysaccharide (LPS), carbon tetrachloride (CCl_4) and silymarin were purchased from Sigma–Aldrich Co. (Shanghai, China). The kits for determining serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were obtained from the Biosino Biotechnology Company Ltd. (Beijing, China). Assay kits used for determination of malondialdehyde (MDA) content, superoxide dismutase (SOD) activity and glutathione peroxidase (GPx) activity were obtained from the Jiancheng Institute of Biotechnology (Nanjing, China). The tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) assay kits were from the Academy of Military Medical Sciences (Beijing, China). All other chemicals used were of analytical grade.

2.2. Plant material and preparation of extract

Artemisia absinthium L. is a perennial subshrub belonging to family Asteraceae. This aromatic plant is up to 1 m tall, having many branching silvery grey stems with small silky pubescent leaves, silvery grey in colour and 2–3 times pinnately divided. Numerous small, yellow, almost spherical flowering heads are arranged in much branched panicles (Wichtl, 1994). The flowering aerial parts of *A. absinthium* L. were purchased from Traditional Uighur Medicine Hospital in Urumqi, Xinjiang Autonomous Region, China, and authenticated by associate pharmacist Gulnar Dawut at the Uighur Medicine Preparation Centre of Traditional Uighur Medicine Hospital, where the voucher specimens have been deposited and registered under number UMHCY 070812. The aqueous extract was prepared from plant material in the traditional manner, as commonly used in Traditional Uighur Medicine. Besides, due to the content of toxic compounds in *A. absinthium* (primarily thujone) which are mainly lipophilic in nature, water is the most suitable solvent since it reduces the thujone content in the extracts to the lowest level (Tegtmeier and Harnischfeger, 1994). Briefly, powdered aerial parts (500 g) were macerated with distilled water (5 l) at room temperature for 12 h, and then boiled for 1 h. After filtration, the extract was dried into the powder by a vacuum-drier at 60 °C. The yield obtained was 25.6% (w/w) relative to the original dry plant material. The extract was suspended in 0.5% sodium carboxymethylcellulose (CMC) solution prior to pharmacological study, and concentrations used in the experiments were based on the dry weight of the extract. The doses of the extract were calculated according to the standard dose of *A. absinthium* extract used in Traditional Uighur Medicine clinical practice, applying appropriate methods for the translation to animals (Reagan-Shaw et al., 2008), and taking into consideration the data of safety tests in the same time (Gilani and Janbaz, 1995; Muto et al., 2003).

2.3. Qualitative and quantitative chemical analysis of the extract

Qualitative characterization of aqueous extract of *A. absinthium* (AEAA) was performed by means of thin layer chromatography (TLC). TLC analyses were carried out on pre-coated silica gel plates (Kieselgel 60 F254, Merck, Germany), using the mobile phases and spray reagents for detection of sesquiterpene lactones (chlorophorm–methanol 95:5; Lieberman–Burchard reagent) as well as flavonoids and phenolic acids (ethyl acetate–formic acid–acetic acid–water 100:11:11:27; Natural product reagent/PEG). Detection was done under UV at 365 nm (Wagner et al., 1983).

For the quantitative analysis of total phenol, tannin, phenolic acid and flavonoid contents in AEAA, the measurements were carried out using UV/VIS Spectrophotometer (Unicam Helios α, Cambridge, UK). Total tannin as well as total phenol contents in AEAA were estimated by a colourimetric assay based on the procedures described in European Pharmacopoeia (2004a). Briefly, 0.500 g of the extract was heated in a water bath with 150 ml of water, at 100 °C for 30 min, then filtrate was filled up to 250 ml with water and obtained solution served as stock solution. An aliquot of stock solution was mixed with phosphomolybdotungstic reagent and sodium carbonate solution. After 30 min, the absorbance was read at 760 nm (A_1), and the quantification of total phenols was done with respect to the standard calibration curve of pyrogallol (6.25–50.00 mg). For the determination of tannin content, stock solution was vigorously shaken with hide powder for 60 min. Since the hide powder adsorbed tannins, phenols unadsorbed on hide powder were measured in filtrate, after addition of phosphomolybdotungstic reagent in a sodium carbonate medium (A_2). The percentage content of tannins, expressed as pyrogallol, was calculated from the following equation: $3.125 \times (A_1 - A_2)/(A_3 \times m)$, where A_3 is the absorbance of the test solution containing 0.050 g of pyrogallol, and m the mass of the extract (g).

Determination of phenolic acid content in AEAA was performed using the method described in European Pharmacopoeia (2004b). To 0.300 g of the extract was added 95 ml of 50% ethanol (v/v) and the mixture was boiled in a water bath for 30 min. Phenolic acids in the filtrate were measured spectrophotometrically, using the nitrite–molybdate reagent of Arnow, in a sodium hydroxide medium, and the percent of total phenolic acids content, expressed as chlorogenic acid, was calculated from the expression: $A \times 5.3/m$, where A is the absorbance of the test solution at 525 nm and m the mass of the extract, in grams.

Quantitative determination of flavonoids in AEAA was performed using spectrophotometric method (Jurišić Grubešić et al., 2007). 1 ml of 0.5% hexamethyl tetramine solution, 20 ml of acetone, and 2 ml of 25% hydrochloric acid were added to the extract