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Original article

Inulin-type fructan and infusion of *Artemisia vulgaris* protect the liver against carbon tetrachloride-induced liver injury

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ABSTRACT

Background: Infusions of aerial parts of *Artemisia vulgaris* L. (Asteraceae) are used in herbal medicine to treat several disorders, including hepatosis.
Purpose: Evaluation of *in vivo* hepatoprotective effects of *A. vulgaris* infusion (VI) and inulin (VPI; *i.e.*, the major polysaccharide of VI).
Study design: The hepatoprotective effect of *A. vulgaris* extracts on carbon tetrachloride (CCl_4)-induced hepatotoxicity and the probable mechanism involved in this protection were investigated in mice.
Methods: *A. vulgaris* infusion (VI) was prepared according to folk medicine using the aerial parts of the plant. Carbohydrate, protein, and total phenolic content was determined in VI, and its phenolic profile was determined by high-performance liquid chromatography (HPLC). Male Swiss mice were orally pretreated for 7 days with VI or VPI (once per day). On days 6 and 7 of treatment, the mice were intraperitoneally challenged with CCl_4. Liver and blood were collected and markers of hepatic damage in plasma and oxidative stress in the liver were analyzed. Hepatic histology and inflammatory parameters were also studied in the liver. The scavenging activity of VI and VPI were evaluated *in vitro* using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.
Results: VI contained 40% carbohydrates, 2.9% proteins and 9.8% phenolic compounds. The HPLC fingerprint analysis of VI revealed chlorogenic, caffeic and dicaffeoylquinic acids as major low-molar-mass constituents. Oral pretreatment with VI and VPI significantly attenuated CCl_4-induced liver damage, reduced the activity of alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) in plasma, and prevented reactive oxygen species accumulation and lipid peroxidation in the liver. Comparisons with the CCl_4-treated group showed that VI and VPI completely prevented necrosis, increased the levels of reduced glutathione (GSH), and reduced tumor necrosis factor alpha (TNF-α) level in the liver. VI and VPI also exhibited high radical scavenging activity *in vitro*.
Conclusion: VI and VPI had remarkable hepatoprotective effects *in vivo*, which were likely attributable to antioxidant and immunomodulatory properties. The present findings support the traditional use of *A. vulgaris* infusion for the treatment of hepatic disorders.

Introduction

Liver diseases are an important problem worldwide. These diseases are caused by several factors such as, alcoholism, xenobiotics, viral infections and drugs (Ingawale et al., 2014). Drug-induced liver injury is a common and important cause of liver injury, comprising half of the cases of acute liver failure. Drug metabolism occurs primarily in the liver, and drug metabolites can cause oxidative stress in hepatocytes, leading to cell death (Kaplowitz, 2004). Conventional drugs that are used for the treatment of hepatic injury have limited therapeutic actions and sometimes adverse effects (Liu et al., 2015). In this context, some extracts and polysaccharides isolated from plants have been reported as potent

Abbreviations: VI, *Artemisia vulgaris* infusion; VPI, polysaccharide from *Artemisia vulgaris* infusion.

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hepatoprotective agents against chemically induced liver injury (Rofiee et al., 2015; Xiao et al., 2012).

In this context, *Artemisia vulgaris* L., commonly known as "mugwort" (family: Asteraceae), is widely known for its medicinal properties. Infusions of its aerial parts are popularly used to treat several disorders, including hepatosis (Govindaraj et al., 2008; Saleh et al., 2014). To date, only one study has reported the hepatoprotective activity of *Artemisia vulgaris*. Gilani et al. (2005) described protective effects of an aqueous-methanolic extract of aerial parts of *A. vulgaris* against D-galactosamine- and lipopolysaccharide-induced hepatitis in mice. However, in folk medicine, infusions of the aerial parts of *A. vulgaris* are used, and their effects on the liver can be different from those of aqueous-methanolic extracts. To our knowledge, the chemical composition of this infusion has not been reported yet.

With regard to the chemical composition of the aerial parts of *A. vulgaris*, the essential oil has been widely studied. The main components were reported to be 1,8-cineole, β-thujone, caryophyllene, germacrene D, and camphor (Govindaraj et al., 2008; Saleh et al., 2014). Polar extracts of this plant contain mainly phenolic compounds, including flavonoids (isoquercitrin, quercitrin, quercetin, luteolin, and kaempferol), hydroxycinnamic acids (gentisic, caffeic, *p*-coumaric, and ferulic acids), and several quinic acid derivatives (3-caffeoylquinic, chlorogenic, 5-feruloylquinic, 3,4-dicaffeoylquinic, 3,5-dicaffeoylquinic, 1,5-dicaffeoylquinic, 1,3-dicaffeoylquinic, 1,4-dicaffeoylquinic and 4,5-dicaffeoylquinic acids) (Ivanescu et al., 2010; Melguizo-Melguizo et al., 2014). However when an infusion is prepared, using hot water, the volatile oil is lost, leaving metabolites as non-volatile compounds and polysaccharides.

Inulin-type fructan is the major polysaccharide of *A. vulgaris* infusions prepared according to methods of traditional medicine (Corrêa-Ferreira et al., 2014). Structurally, inulin is a linear polymer that is constituted by fructose in β-(2→1)-linkages with a starting α-D-glucose unit. Inulin has been widely studied for its prebiotic effects, but other biological properties have also been described, such as hepatoprotective effects (Liu et al., 2015).

In the present study, we investigated the hepatoprotective and antioxidant effects of an *A. vulgaris* infusion and inulin (*i.e.*, the major polysaccharide of *A. vulgaris*) using carbon tetrachloride (CCl_4) to induce hepatic injury in mice. The low-molar-mass compounds that are present in *A. vulgaris* infusion were also investigated.

Materials and methods

Chemicals

Methanol and glacial acetic acid (HPLC grade) were purchased from J. T. Baker (Mexico City, Mexico). Ultra-pure water was obtained from Milli-Q system. Gallic acid, 4-hydroxybenzoic acid, chlorogenic acid, caffeic acid, acetylsalicylic acid, *p*-coumaric acid, benzoic acid, salicylic acid, cinnamic acid, and kaempferol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methyl chlorogenate, 3,4-dicaffeoilquinic acid, 3,5-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid, methyl 3,4-dicaffeoylquinate, methyl 3,5-dicaffeoylquinate, and ethyl caffeate were previously isolated from *Moquiniastrum polymorphum* (Strapasson et al., 2014). 2,2-diphenyl-1-picrylhydrazyl (DPPH), tetramethylbenzidine, *p*-nitrophenyl-2-acetamide-β-D-glucopyranoside, citric acid, glycine, N-1-napthylethylenediamine dihydrochloride (NED), Triton X-100, reduced glutathione (GSH), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), 1-chloro-2,4-dinitrobenzene (CDNB), *O*-cresolsulfonphthalein-3′,3″-bis(methyliminodiacetic acid sodium salt) (Xylenol orange), Coomassie Brilliant Blue G-250, ethylenediaminetetraacetic acid disodium salt (EDTA), and 2′,7′-dichlorodihydrofluorescin diacetate (DCFH-DA) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ascorbic acid, hydrogen peroxide, sodium acetate, sodium phosphate, potassium phosphate, and pyrogallol were obtained from Vetec (Rio de Janeiro, Brazil). Aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) detection kits were purchased from Kovalent (São Gonçalo, Brazil). The tumor necrosis factor α (TNF-α) detection kit was purchased from R&D Systems (Minneapolis, MN, USA). The interleukin 1β (IL-1β) detection kit was purchased from eBioscence (San Diego, CA, USA). Sulfuric acid, bovine serum albumin (BSA), and butylated hydroxytoluene (BHT) were obtained from Merck (Darmstadt, Germany). Ethanol, dimethylformamide, and phosphoric acid were purchased from Neon (São Paulo, Brazil). Tris-HCl and *p*-nitrophenol were obtained from Fluka (St. Louis, MO, USA). Carbon tetrachloride (CCl_4) was obtained from Reagen (Rio de Janeiro, Brazil). Folin-Ciocalteu was obtained from Polipur (Porto Alegre, Brazil). Ketamine was obtained from Vetnil Industry and Trade of Veterinary Products (Louveira, Brazil). Xylazine was obtained from Syntec (Cotia, Brazil). Methanol was obtained from Biotec (Pinhais, Brazil). Ammonium ferrous sulfate and *sulfanilamide were obtained from Synth (Diadema, Brazil).*

Plant material and extract preparation

Aerial parts of *A. vulgaris* were collected in Mandirituba, Paraná, Brazil (April/2014). The plant was identified by Osmar dos Santos Ribas, and a voucher specimen (MBM 388,303) of *A. vulgaris* was deposited at the Herbarium of Museu Botânico Municipal de Curitiba.

The *A. vulgaris* infusion was prepared using the aerial parts of the plant according to traditional methods. One teaspoon of herb (1.4 g) was added to 1 cup (200 ml) of boiling water (Lorenzi and Matos, 2008). The material was infused until it reached 40 °C, and then the extract was filtered using filter paper with pore size 14 µm (Qualy, São José dos Pinhais, Brazil) and freeze-dried. The infusion lyophilized (VI) was used to biological assays and HPLC analyses. A crude polysaccharide fraction rich in inulin-type fructan (VPI), previously isolated and characterized from the *A. vulgaris* infusion (Corrêa-Ferreira et al., 2014), was also used in the experiments. Briefly, VPI was obtained after the preparation of *A. vulgaris* infusion according to traditional methods, which was filtered. The extract was concentrated and treated with ethanol (4:1, v/v). The material was kept at 4 °C overnight and then the polysaccharide (VPI) was isolated by centrifugation (8000 rpm, 20 min), washed with ethanol, and dried under vacuum.

Characterization of A. vulgaris *infusion*

Total carbohydrates were assayed using the phenol–sulfuric acid method (Dubois et al., 1956) with galactose as the standard. Protein content was evaluated according to the Bradford (1976) method with bovine serum albumin (BSA) as the standard. Phenolic content was estimated using Folin–Ciocalteu reagent (adapted from Singleton, et al., 1999) with gallic acid as the standard. The amounts of fructose and fructose-yielding carbohydrates were estimated using a ketose-specific modification of the anthrone method as described by Pollock (1982) with inulin as the standard.

HPLC fingerprint analyses of VI were performed on a Waters HPLC apparatus equipped with 2998 photodiode array detector (PDA). For all of the analyses, a Nucleosil 100-5 C18 column (250 mm × 4.6 mm, 5 mm particle size) was used. The mobile phase consisted of H_2O with 1% acetic acid (A) and methanol (B), applied in a linear gradient from 95:5 (A:B) to 100 (B), over 60 min, followed by isocratic elution with B for 10 min. The flow rate was