Food and Chemical Toxicology 51 (2013) 179–187

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Ethanolic extract of roots from *Arctium lappa* L. accelerates the healing of acetic acid-induced gastric ulcer in rats: Involvement of the antioxidant system

Luisa Mota da Silva [a], Alexandra Allemand [a], Daniel Augusto G.B. Mendes [a], Ana Cristina dos Santos [a], Eunice André [b], Lauro Mera de Souza [c], Thales Ricardo Cipriani [c], Nessana Dartora [c], Maria Consuelo Andrade Marques [a], Cristiane Hatsuko Baggio [a], Maria Fernanda Werner [a,*]

[a] Department of Pharmacology, Federal University of Parana, Curitiba, PR, Brazil
[b] Department of Biophysics and Pharmacology, Federal University of Rio Grande do Norte, RN, Brazil
[c] Department of Biochemistry and Molecular Biology, Federal University of Parana, Curitiba, PR, Brazil

ARTICLE INFO

Article history:
Received 14 December 2010
Accepted 23 September 2012
Available online 2 October 2012

Keywords:
Arctium lappa
Burdock
Acetic acid
Gastric ulcer
Radical scavenger
Inflammation

ABSTRACT

We evaluate the curative efficacy of the ethanolic extract (EET) of roots from *Arctium lappa* (bardana) in healing of chronic gastric ulcers induced by 80% acetic acid in rats and additionally studies the possible mechanisms underlying this action.

Oral administration of EET (1, 3, 10 and 30 mg/kg) reduced the gastric lesion area in 29.2%, 41.4%, 59.3% and 38.5%, respectively, and at 10 mg/kg promoted significant regeneration of the gastric mucosa, which was confirmed by proliferating cell nuclear antigen immunohistochemistry. EET (10 mg/kg) treatment did not increase the gastric mucus content but restored the superoxide dismutase activity, prevented the reduction of glutathione levels, reduced lipid hydroperoxides levels, inhibited the myeloperoxidase activity and reduced the microvascular permeability. In addition, EET reduced the free radical generation and increased scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals *in vitro*. Furthermore, intraduodenal EET (10 and 30 mg/kg) decreased volume and acidity of gastric secretion. Total phenolic compounds were high in EET (Folin–Ciocalteau assay) and the analysis by liquid chromatography-mass spectrometry revealed that the main compounds present in EET were a serie of hydroxycinnamoylquinic acid isomers. In conclusion, these data reveal that EET promotes regeneration of damaged gastric mucosa, probably through its antisecretory and antioxidative mechanisms.

1. Introduction

Gastric ulcer is a benign lesion of the gastric mucosa, which occurs at a site where the mucosal epithelium is exposed to acid and pepsin. Stress, smoking, nutritional deficiencies and ingestion of non-steroidal anti-inflammatory drugs can contribute to increase the incidence of gastric ulcer (Belaiche et al., 2002). In addition, the gastric mucosa has agents like mucus, bicarbonate and prostaglandins that protect the stomach against these lesions (Goel and Bhattacharya, 1991). However, when the superficial levels of mucosal defense fail or are overwhelmed by a luminal insult, an inflammatory response is installed with exposure of tissue to a high oxidative stress, characterizing the onset of the ulcer.

The classic treatment of peptic ulcers includes proton pump inhibitors and H2 receptor antagonists, but clinical evaluation of these drugs has shown incidence of side effects, drug interactions and recurrence after treatments (DeVault and Talley, 2009). The interest in the use of plant extracts in the treatment of several diseases is widespread in many parts of the world, and medicinal plants stand out as important sources of new therapeutic approaches. Thus, identify new potentially agents through natural sources is still essential for more effective and safe antiulcer therapy.

Arctium lappa Linne, popularly known as burdock or bardana, is a member of the Asteraceae (Compositae) family, and its carrot-like root is commonly cooked and eaten as a vegetable in Asia. *A. lappa*, which can be found worldwide, is used therapeutically as diuretic, depurative, digestive stimulant and in dermatological conditions.

Abbreviations: EET, ethanolic extract; DPPH, 2,2-diphenyl-1-picrylhydrazyl; GSH, non-protein sulfhydryl groups; HE, haematoxylin and eosin; LOOH, lipid hydroperoxides; MPO, myeloperoxidase; MVP, microvascular permeability; N, non-ulcerated group; O, omeprazole; PCNA, proliferating cell nuclear antigen; PDA, photodiode array detector; S.E.M., standard error of the mean; SOD, superoxide dismutase; ROS, reactive oxygen species; TAE, tannic acid equivalents; UHPLC–MS, Ultra high performance liquid chromatography–mass spectrometry; VEH, vehicle.

* Corresponding author. Address: Departamento de Farmacologia, Setor de Ciências Biológicas, Universidade Federal do Paraná, Centro Politécnico, Jardim das Américas, P.O. Box 19031, Curitiba, PR, 81530-900, Brazil. Tel.: +55 41 3361 1721; fax: +55 41 3266 2042.

E-mail address: mfernanda.werner@ufpr.br (M.F. Werner).

0278-6915/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fct.2012.09.026

In addition, several investigators have been demonstrated that *A. lappa* displayed hepatoprotective (Lin et al., 2002); antibacterial properties against gram-positive and gram-negative bacteria (Gentil et al., 2006; Pereira et al., 2005) and anti-inflammatory effects (Zhao et al., 2009), which might be due to its free radical-scavenging activity (Leonard et al., 2006).

Our group recently reported that chloroform extract and fractions of *A. lappa* roots shows inhibitory effect on gastric H^+, K^+-ATPase and antioxidant properties that accounts for acid antisecretion and the antiulcer potential of this plant (Dos Santos et al., 2008). Moreover, it is important to note that the chloroform extraction contain mainly non-polar compounds (Dos Santos et al., 2008). It is well know that the type of solvent used in the extraction process determines the chemical composition of the resulting extracts and consequently its biological properties. Thus, considering that in folk medicine *A. lappa* roots is medicinally consumed also as tea form, which contains mainly polar compounds, in the present study we decide to investigate the gastroprotective action of ethanolic extract from *A. lappa* roots, considering that ethanol extraction is able to extract more polar compounds. In light of these considerations, the current study aimed to investigate if the ethanolic extract of *A. lappa*, administered by the oral route, could promote the healing of acetic acid-induced chronic gastric ulcer in rats and additionally to study the possible mechanisms underlying this action.

2. Material and methods

2.1. Chemicals

The following substances were used: Alcian Blue, bovine serum albumin, 2′,7′-dichlorofluorescein-diacetate, 2,2-diphenyl-1-picrylhydrazyl, 5,5′-dithiobis (2-nitrobenzoic acid), evans blue, Folin–Ciocalteu reagent, glutathione, hexadecyltrimethylammonium bromide, omeprazole, pyrogallol, tannic acid, 3,3′,5,5′-tetramethylbenzidine, chlorogenic acid (3-*O*-caffeoylquinic acid) and triton-X (all from Sigma, St. Louis, USA). Absolute ethanol, acetic acid, ascorbic acid, diethyl ether, *N*,*N*-dimethylformamide, formaldehyde, hydrogen peroxide, magnesium chloride, methanol, sodium acetate, sodium carbonate, sucrose, trichloroacetic acid, xylene and xylenol orange (Vetec, Rio de Janeiro, Brazil). The chromatography-grade solvents, methanol and formic acid, were from Tedia, and type 1-deionized water was Milli-Q (Millipore).

2.2. Plant material and preparation of ethanolic extract

Roots of *A. lappa* L. were provided by Chamel Indústria e Comércio de Produtos Naturais Ltda (Campo Largo, Paraná, Brazil), lot 8857. A voucher specimen was deposited in the Herbarium of the Botany Department of the Federal University of Parana, Brazil, under number 37173.

Briefly, the air-dried and grounded roots (7 kg) were defatted in chloroform as previously described (Dos Santos et al., 2008). Then, the residue (6.936 kg) was extracted with 96% ethanol, and held at 25 °C for 24 h. The alcoholic extract was filtered, the ethanol was eliminated by evaporation under reduced pressure and the resulting residue was similarly re-extracted for another 24 h. After eliminating the solvent under reduced pressure, the dried material yielded 210 g (3%) of the crude ethanolic extract (EET).

2.3. Estimation of total phenolic content

Total phenols were determined using the Folin–Ciocalteau reagent, with the microscale protocol developed by (Arnous et al., 2001). About 50, 100, 150 and 200 μg of EET were made up to 0.5 ml with distilled water. The 2.5 ml of Folin–Ciocalteau reagent (1:10 dilution) and 2 ml of sodium carbonate (7.5% w/v) were added and the tubes incubated at 45 °C for 15 min. The absorbance was read at 750 nm using a spectrophotometer and the total polyphenol concentration was calculated from a calibration curve, using tannic acid as a standard. Results were expressed as tannic acid equivalents (TAE) in μg.

2.4. Ultra high performance liquid chromatography–mass spectrometry (UHPLC–MS)

The chromatography was carried out in an Acquity-UPLC® system (Waters, MA, USA), composed by a binary pump, sample manager and column oven, equipped with a photodiode array detector (PDA) and a triple quadrupole, electrospray ionization-mass spectrometry (ESI-MS) Quattro LC (Wates, MA, USA). Samples (EET) were held at room temperature (22 °C) and the column oven at 60 °C. The separation was developed on a Waters BEH-Phenyl, with 50 × 2.1 mm length and 1.7 μm particle size, using formic acid (0.1%, v/v – solvent A) and methanol (solvent B) with a flow rate of 300 μl/min. A non-linear gradient, provided by the Empower® software (Waters, MA, USA), was developed by increasing the solvent B, from 0 to 20% in 6 min (curve 5), to 70% in 9 min (curve 7) then to 100% in 11 min (curve 6). The system returned to initial condition (100% A) in 11.5 min and then the column was reconditioned for 3 min. EET was prepared at 2 mg/ml in MeOH–H_2O (1:1, v/v), with 4 μl being the injection volume. The detections were provided by PDA (scanning 200–400 nm) and ESI-MS (scanning *m/z* 100–1000), operating in the negative ion mode. The ESI-MS energies were set at 2.5 kV (on the capillary) and 50 V (on the cone). Tandem-MS was obtained by collision induced dissociation-mass spectrometry (CID-MS), using argon as collision gas, with energies ranging from 20 to 30 eV.

2.5. Animals

Experiments were conducted using adult female Wistar rats weighing 180–200 g, housed at 22 ± 2 °C under a 12-h light/12-h dark cycle (lights on at 06:00 h) and with access to food and water *ad libitum*. They were deprived of food for 16 h prior to experiments. All experiments were previously approved by the Institutional Ethics Committee of the Federal University of Parana (approval certificate 516) and were carried out in accordance with the international standards and the ethical guidelines on animal welfare.

2.6. Acetic acid-induced chronic gastric ulcers

Chronic gastric ulcers were induced in rats by the method of Okabe et al. (1971) with slight modifications. Rats were randomly distributed into eight groups and each group consisted of eight rats. Briefly, animals were anaesthetized with xylazine/ketamine (7.5 mg/kg and 60 mg/kg) by intraperitoneal administration, the abdomen was exposed and 500 μl 80% acetic acid (v/v) was instilled into a glass tube barrel of 6 mm diameter that was applied to the serosal surface of the stomach for 1 min. The acetic acid was removed by aspiration, and the area was washed with sterile saline. On the second day after the ulcer induction, groups of animals were treated with vehicle (water, 1 ml/kg), omeprazole (40 mg/kg, an inhibitor of gastric H^+, K^+-ATPase), ascorbic acid (250 mg/kg, a standard antioxidant) or EET (1, 3, 10, 30 and 100 mg/kg) twice a day for 7 days by gavage. Omeprazole, ascorbic acid and EET were dissolved immediately before oral administration in water. During this period, body weight of all groups was recorded daily to evaluate possible chronic toxicity induced by EET.

2.7. Assessment of gastric ulcers

On the day following the last treatments, animals were sacrificed and their stomachs were removed and opened throughout the great curvature. The total ulcer area (mm^2) was measured as length (mm) × width (mm). Samples of gastric ulcers were fixed in ALFAC solution (85% alcohol 80 °GL, 10% of formaldehyde at 40% and 5% glacial acetic acid), dehydrated with alcohol and xylene and embedded in paraffin wax, sectioned at 5 μm for histological evaluation after hematoxylin/eosin (HE) staining. The gastric sections were observed and photographed under a stereomicroscope with magnification of 25×.

2.8. Induction of hypersecretion by pylorus ligature in rats

A pyloric ligation was carefully performed in fasted rats under anesthesia (Shay et al., 1945). One hour before the surgery, omeprazole (40 mg/kg, p.o.) and immediately thereafter pyloric ligation was performed, vehicle (water at 1 ml/kg intraduodenal – i.d.) or EET (10, 30 and 100 mg/kg, i.d.) was administered. After 4 h of pyloric ligation, the animals were sacrificed, the stomach was opened, and the gastric secretion was collected. Measurements of volume and total gastric acidity were performed immediately after collection as described previously (Baggio et al., 2005).

2.9. Immunohistochemistry

The immunohistochemical methods were made as previously described (Potrich et al., 2010) using proliferating cell nuclear antigen (PCNA) antibody, a cell division marker to evaluate healing potential of EET in acetic acid-induced ulcers. Paraffin-embedded sections were deparaffinized in xylene and hydrated through standard graded ethanol solutions. Sections were rinsed two times for 5 min each in PBS (pH 7.4), incubated in H_2O_2 solution for 10 min to inactivate endogenous peroxides and then heated in citric acid sodium solution in microwave oven at 100 °C to retrieve antigen for 10 min. Blocking of non-specific reaction was performed with blocking solution (1% BSA and 0.3% Triton X-100 in PBS) for 30 min. The sections were then incubated overnight at 4 °C with goat anti-PCNA (at 1:100; Santa Cruz Biotechnology Inc., CA, USA). After that, slides were rinsed in PBS (pH 7.4) and the sections were incubated in secondary antibody at room temperature for 2 h. After washing, the immunoreacted cells were then developed utilizing