JPP Journal of Pharmacy and Pharmacology

JPP 2008, 60: 795–801

Received September 25, 2007
Accepted February 25, 2008
DOI 10.1211/jpp.60.6.0016
ISSN 0022-3573

Gastroprotective activity of the chloroform extract of the roots from *Arctium lappa* L.

Ana C. dos Santos, Cristiane H. Baggio, Cristina S. Freitas, Juliana Lepieszynski, Bárbara Mayer, André Twardowschy, Fabiana C. Missau, Élide P. dos Santos, Moacir G. Pizzolatti and Maria C. A. Marques

Abstract

Arctium lappa L. is used in folk medicine as a diuretic, depurative and digestive stimulant and in dermatological conditions. The objective of this study was to evaluate the effect and the possible mechanisms involved in the gastroprotective effects of a chloroform extract (CE) of the roots from *A. lappa* and its fractions. Oral pretreatment with CE (10, 30 and 100 mg kg^{-1}) significantly reduced gastric lesions induced by ethanol by 61%, 70% and 76%, respectively. Oral administration of CE (100 mg kg^{-1} per day for 7 days) reduced the chronic gastric ulceration induced by acetic acid by 52%. Intraduodenal CE (100, 300 and 600 mg kg^{-1}) reduced the total acidity of gastric secretion by 22%, 22% and 33%, respectively, while i.p. administration (10, 30 and 100 mg kg^{-1}) inhibited total acidity by 50%, 60% and 67%, respectively. In-vitro, CE inhibited H^+, K^+-ATPase activity with an EC50 of 53 μg mL^{-1} and fraction A (30 and 100 μg mL^{-1}) reduced this by 48% and 89%, respectively. CE had no effect on gastrointestinal motility. CE (250 μg mL^{-1}) and fraction B (100 and 250 μg mL^{-1}) had free-radical scavenging ability, inhibiting 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical activity by 50%, 20% and 55%, respectively. Collectively, the results show that the CE protects animals from gastric lesions by reducing gastric acid secretion via inhibition of gastric H^+, K^+-ATPase.

Department of Pharmacology, Sector of Biological Sciences, Universidade Federal do Paraná, Curitiba, PR, Brazil

Ana C. dos Santos, Cristiane H. Baggio, Cristina S. Freitas, Juliana Lepieszynski, Bárbara Mayer, André Twardowschy, Maria C. A. Marques

Department of Chemistry, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil

Fabiana C. Missau, Moacir G. Pizzolatti

Department of Botany, Sector of Biological Sciences, Universidade Federal do Paraná, Curitiba, PR, Brazil

Élide P. dos Santos

Correspondence: Professor Maria Consuelo Andrade Marques, Department of Pharmacology, Universidade Federal do Paraná, Caixa Postal 19031, Centro Politécnico – Jardim das Américas, 81531-990, Curitiba – PR, Brazil.
E-mail: mconsu@ufpr.br

Introduction

In Brazil, *Arctium lappa* L. (Asteraceae), popularly known as 'bardana', is used in folk medicine as a diuretic, depurative and digestive stimulant, and in dermatological conditions. It also possesses antibacterial (Pereira et al 2005; Gentil et al 2006), antioxidant (Leonard et al 2006) and hepatoprotective properties (Lin et al 2000; Lin et al 2002). Sbolli (2003) described a depressant effect of the ethanolic extract of roots from bardana and its chloroform fraction on the central nervous system, suggesting the involvement of the gamma aminobutyric acid (GABA)ergic system. Arctigenin and baicalin, compounds isolated from bardana, have activity against human immunodeficiency virus (HIV), characterized by a reduction in the protein expression of HIV (p17 and p24) and reduction in the reverse transcriptase activity by 80–90% (Schröder et al 1990; Kitamura et al 1998; Wang et al 2004). Kardošová et al (2003) reported mitogenic and co-mitogenic activities for bardana and confirmed the immunobiological activity of its isolated compound, inulin. Chlorogenic and caffeic acids, also present in *A. lappa*, showed protective activities against low-density lipoprotein oxidation (Soliman & Mazzio 1998; Dinis et al 2002). However, so far the gastroprotective effects of the plant have not been investigated. In the present study, we determined whether inhibition of the H^+, K^+-ATPase and scavenging of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical could be involved in the gastroprotective mechanisms of the chloroform extract (CE) of the roots from *A. lappa* L. and its fractions.

Materials and Methods

Plant material

Roots of *A. lappa* L. were provided by the Fazenda Solidariedade, of Fundação de Ação Social (FAS) from their plantation in Curitiba city (PR, Brazil). A specimen is deposited in the Herbarium of the Department of Botany of the Universidade Federal do Paraná (UFPR), under voucher number 37173.

Preparation of the chloroform extract

Air-dried and powdered roots (13 kg) were extracted with chloroform (2×18L) for 24 h at room temperature. The solvent was filtered and evaporated under reduced pressure to dryness, with an extraction yield of 125 g (0.96%) for the crude CE. CE, obtained by using simultaneous derivatization with diazomethane and acetic anhydride/DMAP acetylation, was examined by on-column high-temperature gas chromatography using a DB-1HT fused silica capillary column (15 m length × 0.25 mm ID × 0.1 μm film; Agilent Technologies, Santa Clara, CA, USA). The oven temperature was increased from 80°C to 370°C at a rate of 10°C min^{-1}, with the flame ionization detector (FID) temperature set at 380°C (Figure 1). The CE was subjected to column chromatography over silica gel (70–230 mesh), eluted with mixtures of *n*-hexane/ethyl acetate, ethyl acetate and ethanol. The fractions eluted with *n*-hexane/ethyl acetate 3:1 v/v (fraction A), 1:1 v/v (fraction B), 1:3 v/v (fraction C), EtOAc 100% (fraction D), and EtOH 100% (fraction E) were tested for pharmacological activity. The phytochemical analyses of those fractions were performed by gas chromatography-FID and IR and NMR spectrometry.

Fraction A showed the presence of unsaturated fatty acids and esters of unsaturated fatty acids as the main constituents, together with unidentified minor compounds. In fraction B, a mixture of taraxasterol, stigmasterol and chlorogenic acid was identified, together with unidentified minor compounds. Fraction C was β-sitosterol-β-D-glucoside. NMR analysis of fraction D showed it to be composed of caffeic acid and β-sitosterol β-D-glucoside.

Animals

Female Wistar rats (180–200 g) and female Swiss mice (25–30 g) were from the UFPR colony and were maintained under standard laboratory conditions (12 h light–dark cycle, temperature 22 ± 2°C). Standard pellet food (Nuvital, Curitiba/PR, Brazil) and water were available ad libitum. The animals were fasted for 16 h before experiments. All experimental protocols using animals were performed according to the *Principles of Laboratory Animal Care* (NIH Publication 85-23, revised 1985) adopted by UFPR. The Institutional Ethics Committee of the UFPR approved all procedures used in this study (approval certificate 160).

Induction of acute gastric lesions in rats

Rats (n = 6) were treated orally with vehicle (water and 5% Tween 80, 0.1 mL 100 g^{-1} body weight), CE (10, 30 and 100 mg kg^{-1}) or omeprazole (40 mg kg^{-1}) 60 min before oral administration of 80% ethanol (0.5 mL 200 g^{-1}). Animals were killed by cervical dislocation 1 h after lesion induction (Robert et al 1979). The stomachs were removed and the extent of the gastric lesions measured as:

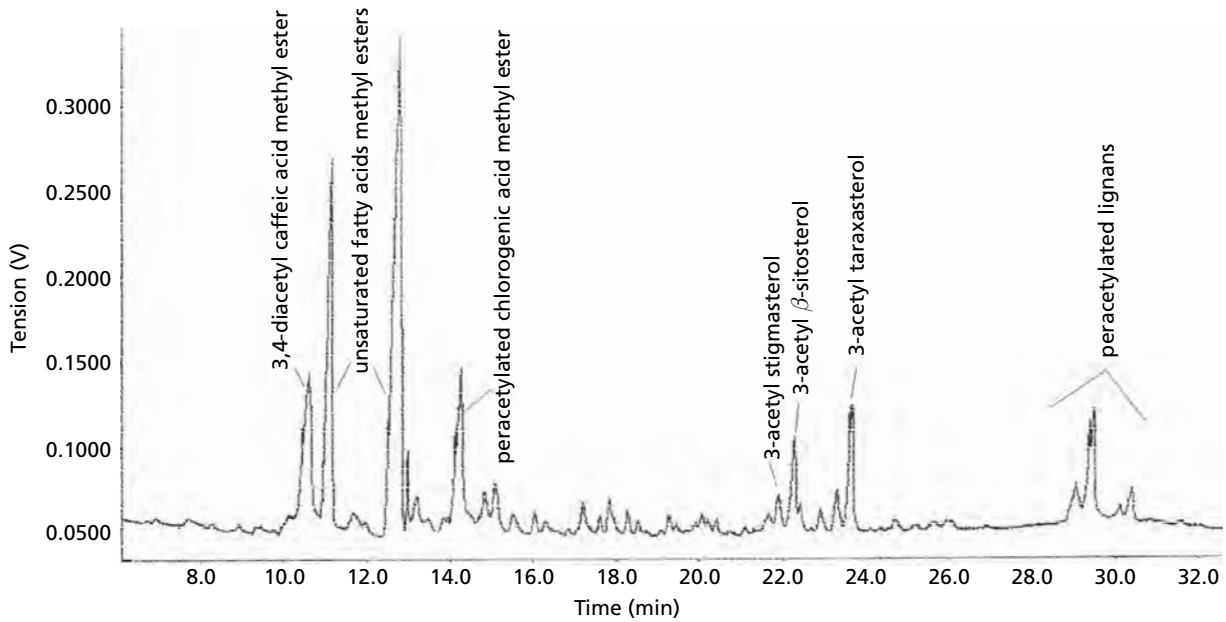

Figure 1 On-column high-temperature gas chromatogram of the chloroform extract from *A. lappa*.

total injured area (mm^2) = length (mm) × width (mm) of injury.

Induction of chronic gastric lesions in rats

Chronic gastric ulcers were induced in rats using acetic acid, according to a modification of the method described by Takagi et al (1969). Briefly, animals were anaesthetized, the abdomen was exposed and 50 μL 20% acetic acid (v/v) was injected into the anterior subserosa, and 50 μL 0.9% saline into the posterior subserosa of the gastric wall. After recovery from anaesthesia, animals were treated orally with vehicle (water and 5% Tween 80, 0.1 mL(100 g body weight)$^{-1}$), CE (10, 30 and 100 mg kg^{-1}) or omeprazole (20 mg kg^{-1}) twice a day for 7 days, starting on the seventh day after injection of acetic acid when ulcers are established. Animals were killed on the day after the last administration. The stomachs were removed and the ulcers separated from the healthy tissue. Ulcer volume (mm^3) was calculated as = length × width × depth (all in mm) of the ulcer.

Induction of hypersecretion by pylorus ligature in rats

Pylorus ligatures (Shay et al 1945) were applied in fasted female rats (n = 6) under ether anaesthesia and CE (100, 300 or 600 mg kg^{-1} intraduodenally (i.d.) or 10, 30 and 100 mg kg^{-1} i.p.), vehicle (water and 5% Tween 80, 0.1 mL (100 g body weight)$^{-1}$, i.d. or i.p.) or omeprazole (40 mg kg^{-1} orally) were administered immediately. After 4 h of the pylorus ligature (2 h in the groups treated i.p.), animals were killed by deep anaesthesia, the stomach was opened and gastric secretion collected. Measurements of volume and total gastric acidity were performed immediately after collection as described previously (Baggio et al 2005).

In-vitro determination of H^+, K^+-ATPase activity

Gastric H^+, K^+-ATPase activity was used as a measure of gastric acid secretion. Gastric H^+, K^+-ATPase was prepared by ultracentrifugation and gradient separation. Briefly, mucosal tissues from the corpus region of a rabbit stomach were scraped, suspended in 10 volumes of the homogenizing buffer (HB: 250 mM sucrose, 2 mM $MgCl_2$, 1 mM EGTA and 2 mM HEPES, pH 7.4 with protease inhibitor (composition in mM, AEBSF 104-pepstatin, aprotinin 0.08-pepstatin, leupeptin 2-pepstatin, bestatin 4-pepstatin A 1.5, E-64 1.4)) and homogenized (20 up-and-down strokes of a motor-driven Teflon pestle (1500 rev min^{-1})), then centrifuged (20000 *g* for 20 min). The supernatant was centrifuged at 100000 *g* for 60 min and the resulting pellet was homogenized in HB, placed on top of a 30% sucrose cushion and centrifuged at 100000 *g* for 2 h. The gastric vesicular fraction containing H^+, K^+-ATPase remaining at the isotonic 30% sucrose interface was collected and stored at −70°C. All procedures were performed at 4°C.

Protein concentration was determined in 96-well plates using a proprietary BCA protein assay kit (Pierce, Rockford, IL, USA), using bovine serum albumin as the standard.

Enzyme activity was determined by incubating 4 μg of protein with/without CE (10–300 μg mL^{-1}) or its fractions (10–500 μg mL^{-1}) in a reaction mixture containing 1 mM ATP, 20 mM KCl, 2 mM $MgCl_2$ and 50 mM Tris HCl (pH 7.4) at 37°C for 20 min (Murakami et al 1992). The reactions were terminated by addition of 60 μL ice-cold 50% trichloroacetic acid. The amount of inorganic phosphate formed was quantified according to the method of Fiske & Subbarow (1925). Activity was calculated using the extinction coefficient of inorganic phosphate (P_i) (ε = 11000 $M^{-1}cm^{-1}$) (Kubo et al 1995; Baggio et al 2005). Experiments were performed in triplicate.

Determination of gastrointestinal motility

Fasted female Swiss mice (n = 8) were treated orally with CE (30, 100 and 300 mg kg^{-1}), metoclopramide (30 mg kg^{-1}) or vehicle (water and 5% Tween 80, 0.1 mL (10 g body weight)$^{-1}$) or subcutaneous atropine (3 mg kg^{-1}) 60 min before oral administration of 0.5 mL of a semisolid solution of 0.05% phenol red and 1.5% methylcellulose. After 15 min, the animals were killed and the stomach and small intestine quickly removed. Determinations were made using a modification of the method described by Suchitra et al (2003). Briefly, gastric emptying (GE) was measured as the amount of marker that remained in the stomach at the end of the experiment. Stomachs were individually homogenized with 7 mL distilled water and centrifuged at 3000 rev min^{-1} for 15 min. Equal amounts (1 mL) of supernatant and 0.025 M NaOH were mixed and the absorbance measured using a spectrophotometer at 560 nm. GE (%) was calculated using the equation %GE = 100 − (X × 100/Y), where X is the absorbance of phenol red recovered from the stomach of animals sacrificed 15 min after the administration of marker, and Y is the mean (n = 8) absorbance of phenol red recovered from the stomachs of control animals (killed immediately after administration of the marker). Intestinal transit (IT) was measured as the distance travelled by the marker in the small intestine. Briefly, the small intestine was dissected from the pylorus to the ileocaecal junction. The total length of the small intestine and the distance travelled by phenol red were then measured. IT was calculated as %IT = X/Y × 100, where X is the distance travelled by phenol red and Y is the total length of the small intestine.

DPPH free-radical scavenging assay

DPPH is a stable free radical that is widely used as a tool to estimate the free-radical scavenging activity of antioxidants. The free-radical scavenging activity of CE and its fractions (50, 100 and 250 μg mL^{-1}) on the DPPH radical was determined using the method described by Blois (1958) and Chen et al (2004), with some modifications. Aliquots of the extract and fractions (0.75 mL) were mixed with 0.25 mL DPPH-radical solution in methanol. The decrease in absorbance at 517 nm was measured at each predetermined checkpoint. For all experiments, the vehicle of the extract and fractions was used as negative control; ascorbic acid (50 μg mL^{-1}) was used as a positive control.

Statistical analysis

Data are expressed as mean ± s.e.m. The significance of the results was determined using one-way analysis of variance followed by the Bonferroni test. Data were considered significant at $P<0.05$. The dose/concentration that produced 50% effect/inhibition (ED50/IC50) were calculated by fitting the data to the equation: $Vi/Vo = 1/(1 + [I]/IC50)$, where Vi = total activity and Vo = remaining activity, using the KhaleidaGraph 3.0 for Windows program (Synergy Software, PA, USA).

Results

Effects on gastric injury

Oral pretreatment with CE at doses of 10, 30 and 100 mg kg^{-1} significantly reduced gastric lesions induced by ethanol, by 61%, 70% and 76%, respectively (control value = 183.0 ± 2.8 mm^2) (Figure 2). Omeprazole, the positive control for the test, reduced gastric lesions by 75%.

Oral administration of CE (100 mg kg^{-1} daily for 7 days) reduced the chronic gastric ulceration induced by acetic acid by 52% compared with the control group (control value = 176.6 ± 17.6 mm^3) (Figure 2B). The positive control for the test, oral omeprazole (20 mg kg^{-1} daily for 7 days), reduced the gastric ulceration index of acetic acid by 71%.

Effects on gastric acid secretion

Intraduodenal administration of CE (600 mg kg^{-1}) immediately after application of the pylorus ligature reduced the volume of the gastric contents secreted over 4 h by 44%. Total acidity was reduced by 22%, 22% and 33% by CE 100, 300 and 600 mg kg^{-1}. Oral omeprazole (40 mg kg^{-1}) reduced the volume by 61% and total acidity by 89%, respectively, (Table 1). CE (30 and 100 mg kg^{-1}) administered i.p. reduced the volume secreted by 54% and 58%, respectively after 2 h of pylorus ligature.

Total acidity of gastric juice was inhibited by 50%, 60% and 67% by CE 10, 30 and 100 mg kg^{-1}, respectively. Omeprazole, the positive control for the test, reduced the volume of gastric acid secretion by 55% and total acidity by 93% (Table 1).

In-vitro, CE potently reduced the hydrolysis of ATP by the rabbit gastric ATPase, with an IC50 of 53 μg mL^{-1} (Figure 3). Fraction A (30 and 100 μg mL^{-1}) reduced the hydrolysis of ATP by the rabbit gastric ATPase by 48% and 89%, respectively (Figure 3). The other fractions of CE did not have any effect on the proton pump (data not shown).

Effects on gastrointestinal motility

GE of semisolid phenol red after 15 min in control mice was 52.6 ± 9.3%. Treatment with oral CE (30, 100 and 300 mg kg^{-1}) did not alter GE. Oral metoclopramide (30 mg kg^{-1}) enhanced GE by 44%. Similarly, oral administration of CE (30, 100 and 300 mg kg^{-1}) did not alter IT (control value = 66.3 ± 2.4%). Atropine, the positive control for the test, reduced IT by 68% (data not shown).

Effects on DPPH scavenging activity

CE (250 μg mL^{-1}) scavenged DPPH radicals, with a decrease in radical activity of 50% when compared with the control (0.200 ± 0.002 nM) (Figure 4), while fraction B (100 and 250 μg mL^{-1}) reduced radical activity by 20% and 55%, respectively (Figure 4). Fractions A, C, D and E showed no scavenging activity (data not shown). Ascorbic acid was used as the reference compound, scavenging 60% and 68% of DPPH radicals.

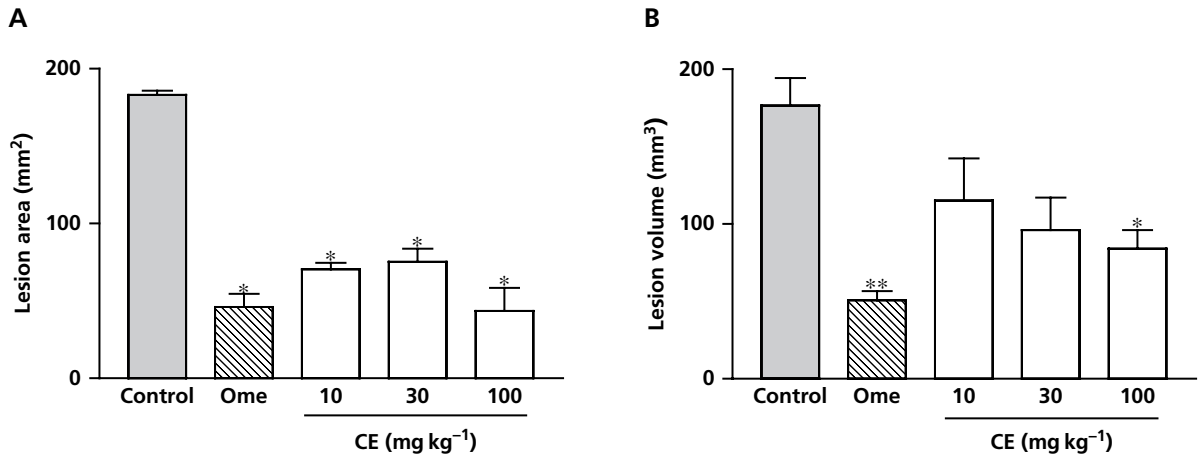

Figure 2 Gastroprotective effects of the chloroform extract (CE) of *A. lappa* administered orally to rats with acute gastric lesions induced by 80% ethanol (A) or chronic gastric ulcers induced by acetic acid (B). Omeprazole (Ome; 40 mg kg^{-1} in A, 20 mg kg^{-1} in B) was used as the positive control. Data are mean ± s.e.m. (n = 6). *$P < 0.05$; **$P < 0.01$ (analysis of variance followed by Bonferroni's test).

Table 1 Effects of the chloroform extract (CE) of *A. lappa* administered i.d. and i.p. on gastric acid secretion in rats with pylorus ligature. Omeprazole was used as a positive control

Treatment	Volume (mL)	Total acidity ($mEq[H^+]\ mL^{-1}$)
i.d. administration		
Control (vehicle) 0.1 mL 100 g^{-1}	11.0 ± 1.2	0.090 ± 0.004
Omeprazole (40 mg kg^{-1} p.o.)	4.4 ± 0.6*	0.011 ± 0.003*
CE 100 mg kg^{-1}	9.4 ± 0.9	0.070 ± 0.003*
CE 300 mg kg^{-1}	9.7 ± 0.7	0.069 ± 0.003*
CE 600 mg kg^{-1}	6.9 ± 0.8*	0.070 ± 0.004*
i.p. administration		
Control (vehicle) 0.1 mL 100 g^{-1}	4.9 ± 0.3	0.030 ± 0.006
Omeprazole (40 mg kg^{-1} p.o.)	3.6 ± 0.2*	0.002 ± 0.001*
CE 10 mg kg^{-1}	3.9 ± 0.3	0.015 ± 0.003*
CE 30 mg kg^{-1}	3.6 ± 0.3*	0.012 ± 0.002*
CE 100 mg kg^{-1}	3.3 ± 0.1*	0.010 ± 0.002*

*$P < 0.05$ vs control.

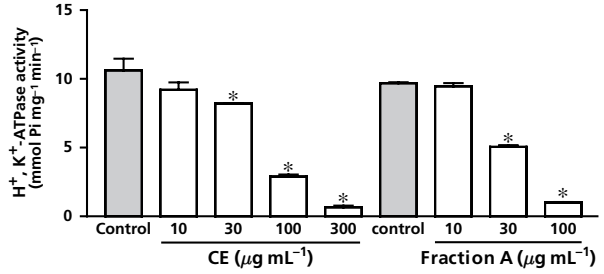

Figure 3 Inhibition of H^+, K^+-ATPase activity in-vitro by the chloroform extract (CE; 10–300 μg mL^{-1}) and fraction A from *A. lappa* (10–300 μgmL^{-1}), measured as formation of inorganic phosphate (Pi). Data are mean ± s.e.m. from triplicate experiments. *$P < 0.05$ (analysis of variance followed by Bonferroni's test).

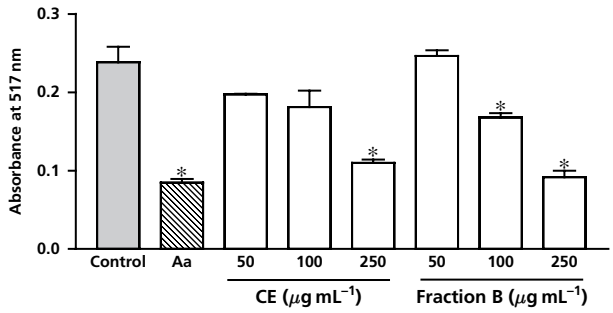

Figure 4 DPPH scavenging activity of the chloroform extract (CE; 50–250 μgmL^{-1}) and fraction B (50–250 μgmL^{-1}) from *A. lappa*. Ascorbic acid (Aa; 50 μgmL^{-1}) was used as a positive control. Data are mean ± s.e.m. from triplicate experiments. *$P < 0.05$ (analysis of variance followed by Bonferroni's test).

Discussion

In this study, we show that the CE from the roots of *A. lappa* effectively protects animals from acute and chronic experimental gastric lesions; this protection appears to be correlated with inhibition of gastric acid secretion.

Acute gastric injury induced in rats by the necrotizing agent ethanol was potently decreased by pretreating animals with the CE. Ethanol is a well-known necrotizing agent that destroys the protective factors of the mucosa (Wallace & Ma 2001), decreasing the mucous barrier (Hirschowitz 1983), gastric mucosal blood flow (reviewed by Abdel-Salam et al 2001) and levels of glutathione, a free-radical scavenger of the gastric mucosa (Repetto & Llesuy 2002). The destruction of these factors promotes haemorrhage, resulting in injury by oxidative damage to gastric epithelial cells. This damage leads to the constriction of veins and arteries of the gastric mucosa, producing congestion, inflammation and tissue injury (Repetto & Llesuy 2002). In our experiments, none of these protective factors were altered by treatment with CE, which indicates that its protective action against ethanol injury is not related to any of these important mechanisms of cytoprotection (data not shown).

The healing effect of the CE in the model of chronic gastric ulceration induced by acetic acid shows its potential use for the treatment of gastric ulcers. Several mechanisms have been reported in the process of healing of gastric ulcers, including stimulation of expression of epidermal growth factor by capsaicin-sensitive nerves, enhancement of mucus production and production of cyclooxygenase-2 derived prostaglandins, and inhibition of neutrophil infiltration and lipid peroxidation in ulcerated gastric tissues (Rodriguez et al 2003). Healing also requires angiogenesis in the granulation tissue at the base of the ulcer and replication of epithelial cells at the ulcer margins, to re-establish the glandular architecture (Wallace 2005). Oxygen-derived free radicals have recently been postulated to play an important role in the pathogenesis of acute gastric mucosal injury induced by ethanol in rats. Furthermore, it has been suggested that free radicals generated by neutrophils may be important factors in delaying the healing of acetic-acid-induced chronic gastric ulcers in these animals (Hamaishi et al 2006). It has been reported that inhibition of enhanced lipid peroxidation in ulcerated gastric tissues by the administration of various antioxidants and superoxide dismutase promotes the healing of acetic-acid-induced chronic gastric ulcers in rats (Kobayashi et al 2001). In our experiments, CE was shown to possess one of these protective factors: antioxidant activity. However, we cannot discount the possibility that the radical-scavenging activity of CE plays an important role in its gastroprotective effect.

Another important protective factor is the inhibition of acid secretion, the main factor responsible for the occurrence of the ulcer (Konturek et al 2005). Thus, CE showed a similar pattern of antiulcer activity to that observed for the positive control for the test, omeprazole, which is a potent inhibitor of gastric acid secretion via irreversible inhibition of H^+, K^+-ATPase activity. When CE was administered by the i.d. route in the pyloric-ligature-induced hypersecretion model, we observed a slight inhibition in the total acidity of gastric secretion. CE was a potent inhibitor of H^+, K^+-ATPase in-vitro. This led us to hypothesize that the effect of CE on gastric secretion may be altered depending on the route of administration. We therefore tested the i.p. administration of CE in the same model, and observed a potent decrease in the

total acidity of gastric secretion. These findings suggest that CE could be inactivated by hepatic metabolism, reducing its bioavailability.

Our in-vitro studies showed that inhibition of gastric acid secretion occurs by inhibition of H^+, K^+-ATPase, which is the final step in acid secretion and therefore one of the most important steps in this process. The antisecretory activity of CE does not totally explain the potent gastroprotective effect observed in the ethanol- and acetic-acid-induced ulcers. Although at this point we cannot discount the possibility that these or other factors involved in the repair of tissue injury may be activated by the CE, it is clear that inhibition of gastric acid secretion (at least partially) and the antioxidant activity of CE play important roles in the gastroprotective properties of *A. lappa*.

In the gastrointestinal motility model, the GE rate is related to a neurohumoral mechanism, which depends on an intact vagal innervation. Thus, the myenteric neurons of the gastric wall are subject to the action of several neurotransmitters, such as acetylcholine (ACh) (reviewed by Hansen 2003). ACh, described as the major regulator of gastrointestinal motility, is the endogenous neurotransmitter at cholinergic synapses in the central and peripheral nervous systems. The stimulation of vagal input to the gastrointestinal tract increases tone, amplitude of contraction and secretory activity of the stomach and intestine (Sagar et al 2005). The excitatory neurons contain ACh and/or substance P, and project to the circular layer of the gastrointestinal wall, promoting contraction (reviewed by Hansen 2003). CE did not alter gastrointestinal motility, suggesting that the cholinergic pathway is not involved in the gastroprotective action of CE.

To identify the probable active principle(s) responsible for the gastroprotective activity of CE, a bioguided purification of the extract was performed, and the fractions obtained were tested in-vitro. Fraction A was the only fraction that inhibited H^+, K^+-ATPase activity. It is the least purified fraction chemically speaking, and contains a mixture of compounds (the greater part being unsaturated fatty acids and esters of the unsaturated fatty acids), which may explain the effect; by contrast, the other fractions were more pure. These unsaturated fatty acids may promote the effect of inhibition of proton pump activity by interaction with the components of the proton pump in the membrane.

Fraction B had DPPH-radical scavenging effects, whereas the other fractions did not show any antioxidant effect. Some compounds present in *A. lappa*, such as chlorogenic and caffeic acids, have been reported to have antioxidant activity. Caffeic acid has antioxidant capacity and free-radical scavenging activity (Mahakunakorn et al 2004; Kang et al 2006). Marked antioxidant activity of chlorogenic acid and caffeic acid have also been observed in-vitro (Chen et al 2004; Wang et al 2006). Chlorogenic acid directly interacts with reactive oxygen species and it is reported to be an effective hydroxyl-radical scavenger. The antioxidant property of chlorogenic acid is attributed to the catechol structure of the phenyl ring, and the double bond conjugated with the catechol group may also serve as a site for free-radical attack (Shi et al 2007). These data suggest that the chlorogenic acid contained in fraction B is the active principle responsible for the antioxidant activity. Although caffeic acid was detected in fraction D, this fraction did not show any antioxidant activity. This is perhaps due to its low concentration and the fact that it is associated with β-sitosterol β-D-glucoside, which is a major constituent of this fraction. Unfortunately, the yield of all these fractions was too low for us to carry out in-vivo experiments to confirm their activities.

Conclusions

Collectively, the results show that the CE of roots from *A. lappa* elicits gastroprotective effects by inhibition of gastric acid secretion (at least partially), which seems to result from inhibition of gastric H^+, K^+-ATPase. However, we cannot discount the possibility that the antioxidant activity of CE also plays an important role in the gastroprotective effects. These findings, corroborating the use of this agent in folk medicine, indicate that *A. lappa* displays good anti-ulcer activity, contributing to its pharmacological validation.

References

Abdel-Salam, O. M., Czimmer, J., Debreceni, A., Szolcsányi, J., Mózsik, G. (2001) Gastric mucosal integrity: gastric mucosal blood flow and microcirculation. An overview. *J. Physiol. Paris* **95**: 105–127

Baggio, C. H., Otofuji, G. M., Souza, W. M., Santos, C. A. M., Torres, L. M. B., Rieck, L., Marques, M. C. A., Mesia-Vela, S. (2005) Gastroprotective mechanisms of indole alkaloids from *Himatanthus lancifolius*. *Planta Med*. **71**: 733–738

Blois, M. S. (1958) Antioxidant determinations by the use of a stable free radical. *Nature* **181**: 1199–1200

Chen, F. A., Wu, A. B., Chen, C. Y. (2004) The influence of treatments on the free radical scavenging activity of burdock and variations of its active. *Food Chem*. **86**: 479–484

Dinis, T. C., Santosa, C. L., Almeida, L. M. (2002) The apoprotein is the preferential target for peroxynitrite-induced LDL damage protection by dietary phenolic acids. *Free Radic. Res*. **36**: 531–543

Fiske, C. H., Subbarow, Y. (1925) The colorimetric determination of phosphorus. *J. Biol. Chem*. **66**: 375–400

Gentil, M., Pereira, J. V., Sousa, Y. T. C. S., Pietro, R., Neto, M. D. S., Vansan, L. P., França, S. C. (2006) In vitro evaluation of the antibacterial activity of *Arctium lappa* as a phytotherapeutic agent used in intracanal dressings. *Phytother. Res*. **20**: 184–186

Hamaishi, K., Kojima, R., Ito, M. (2006) Anti-ulcer effect of tea catechin in rats. *Biol. Pharm. Bull*. **29**: 2206–2213

Hansen, M. B. (2003) Neurohumoral control of gastrointestinal motility. *Physiol. Res*. **52**: 1–30

Hirschowitz, B. I. (1983) Neural and hormonal control of gastric secretion. *Am. Physiol. Soc*. **127**: 128–158

Kang, K. A., Lee, K. H., Zhang, R., Piao, M., Chae, S., Kim, K. N., Jeon, Y. J., Park, D. B., You, H. J., Kim, J. S., Hyun, J. W. (2006) Caffeic acid protects hydrogen peroxide induced cell damage in wi-38 human lung fibroblast cells. *Biol. Pharm. Bull*. **29**: 1820–1824

Kardošová, A., Ebringerová, A., Alföldi, J., Nosál'ová, G., Franová, S., Hríbalová, V. (2003) A biologically active fructan from the roots of *Arctium lappa* L., var. Herkules. *Int. J. Biol. Macromol*. **33**: 135–140

Kitamura, K., Honda, M., Yoshizaki, H., Yamamoto, S., Nakane, H., Fukushima, M., Ono, K., Tokunaga, T. (1998) Baicalin, an inhibitor of HIV-1 production in vitro. *Antiviral Res*. **37**: 131–140

Kobayashi, T., Ohta, Y., Yoshino, J., Nakazawa, S. (2001) Teprenone promotes the healing of acetic acid-induced chronic

gastric ulcers in rats by inhibiting neutrophil infiltration and lipid peroxidation in ulcerated gastric tissues. *Pharmacol. Res*. **43**: 23–30

Konturek, S. J., Konturek, P. C., Brzozowski, T., Konturek, J. W., Pawlik, W. W. (2005) From nerves and hormones to bacteria in the stomach; Nobel prize for achievements in gastrology during last century. *J. Physiol. Pharmacol*. **56**: 507–530

Kubo, K., Uehara, A., Kubota, T., Nozu, T., Moriya, M., Watanabe, Y., Shoji, E., Santos, S. B., Harada, K., Kohgo, Y. (1995) Effects of ranitidine on gastric vesicles containing H^+/K^+-adenosine triphosphatase in rats. *Scand. J. Gastroenterol*. **30**: 944–951

Leonard, S. S., Keil, D., Mehlman, T., Proper, S., Shi, X., Harris, G. K. (2006) Essiac tea: scavenging of reactive oxygen species and effects on DNA damage. *J. Ethnopharmacol*. **103**: 288–296

Lin, S.C., Chung, T. C., Lin, C. C., Ueng, T. H., Lin, Y. H., Lin, S. Y., Wang, L. Y. (2000) Hepatoprotective effects of *Arctium lappa* on carbon tetrachloride- and acetaminophen-induced liver damage. *Am. J. Chin. Med*. **28**: 163–173

Lin, S. C., Lin, C. H., Lin, C. C., Lin, Y. H., Chen, C. F., Chen, I. C., Wang, L. Y. (2002) Hepatoprotective effects of *Arctium lappa* Linne on liver injuries induced by chronic ethanol consumption and potentiated by carbon tetrachloride. *J. Biomed. Sci*. **9**: 401–409

Mahakunakorn, P., Tohda, M., Murakami, Y., Matsumoto, K., Watanabe, H. (2004) Antioxidant and free radical-scavenging activity of choto-san and its related constituents. *Biol. Pharm. Bull*. **27**: 38–46

Murakami, S., Arai, I., Muramatsu, M., Otomos, S., Baba, K., Kido, T., Kozawa, M. (1992) Inhibition of gastric H^+/K^+-ATPase and acid secretion by cassigarol A: a polyphenol from *Cassia garrettiana* Craib. *Biochem. Pharmacol*. **44**: 33–37

Pereira, J. V., Bergamo, D. C. B., Pereira, J. O., França, S. C., Pietro, R., Sousa, Y. T. C. S. (2005) Antimicrobial activity of *Arctium lappa* constituents against microorganisms commonly found in endodontic infections. *Braz. Dent. J*. **16**: 192–196

Repetto, M. G., Llesuy, S. F. (2002) Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. *Braz. J. Med. Biol. Res*. **35**: 523–534

Robert, A., Nezamis, J. E., Lancaster, C., Hauchar, A. J. (1979) Cytoprotection by prostaglandins in rats. Prevention of gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl and thermal injury. *Gastroenterology* **77**: 433–443

Rodriguez, J. A., Astudillo, L., Schmeda-Hirschmann, G. (2003) Oleanolic acid promotes healing of acetic acid-induced chronic gastric lesions in rats. *Pharmacol. Res*. **48**: 291–294

Sagar, L., Sehgal, R., Ojha, S. (2005) Evaluation of antimotility effect of *Lantana camara* L. var. *acuelata* constituents on neostigmine induced gastrointestinal transit in mice. *BMC Complement. Altern. Med*. **5**: 18

Sbolli, K. C. (2003) *Arctium lappa*: Efeitos depressores do sistema nervoso central em roedores. Curitiba. Dissertação (Mestrado em Farmacologia) – Setor de Ciências Biológicas, Universidade Federal do Paraná

Schröder, H. C., Merz, H., Steffen, R., Muller, W. E., Sarin, P. S., Trum, M. S., Schulz, J., Eich, E. (1990) Differential in vitro anti-HIV activity of natural lignans. *Z. Naturforsch*. **45**: 1215–1221

Shay, H., Komarov, S. A., Fels, S. S., Meranze, D., Gruenstein, M., Siplet, H. (1945) A simple method for the uniform production of gastric ulceration in rat. *Gastroenterology* **5**: 43–61

Shi, G. R., Rao, L. Q., Yu, H. Z. (2007) Yeast-cell-based microencapsulation of chlorogenic acid as a water-soluble antioxidant. *J. Food Engineer*. **80**: 1060–1067

Soliman, K. F., Mazzio, E. A. (1998) In vitro attenuation of nitric oxide production in C6 astrocyte cell culture by various dietary compounds. *Proc. Soc. Exp. Biol. Med*. **218**: 390–397

Suchitra, A. D., Dkhar, S. A., Shewade. D.G., Shashindran, C. H. (2003) Relative efficacy of some prokinetic drugs in morphine-induced gastrointestinal transit delay in mice. *World J Gastroenterol*. **9**: 779–783

Takagi, E., Okabe, S., Saziki, R. (1969) A new method for the production of chronic gastric ulcer in rats and the effect of several drugs on healing. *Jpn J. Pharmacol*. **19**: 416–426

Wallace, J. L. (2005) Recent advances in gastric ulcer therapeutics. *Curr Opin Pharmacol*. **5**: 573–577

Wallace, J. L., Ma, L. (2001) Inflammatory mediators in gastrointestinal defense injury. *Exp. Biol. Med*. **226**: 1003–1015

Wang, Q., Wang, Y. T., Pu, S. P, Zheng, Y. T. (2004) Zinc coupling potentiates anti-HIV-1 activity of baicalin. *Biochem. Biophys. Res. Commun*. **324**: 605–610

Wang, B. S., Yen, G. C., Chang, L. W., Yen, W. J., Duh, P. D. (2006) Protective effects of burdock (*Arctium lappa* Linne) on oxidation of low-density lipoprotein and oxidative stress in RAW 264.7 macrophages. *Food Chem*. **101**: 729–738