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Full Length Research Paper

Antiulcerogenic activity of aqueous fraction from leaves of *Arctium lappa* L. (Asteraceae)

Ana Beatriz Albino de Almeida[1], Anderson Luiz-Ferreira[1]*, Maíra Cola[1], Leônia Maria Batista[2], Luciana Di Pietro Magri[1], Márcio Ricardo Fornazari Tiene[3], Lourdes Campaner Santos[3], Wagner Vilegas[3] and Alba R. M. Souza-Brito[1]

[1]Departamento de Anatomia, Biologia Celular e Fisiologia, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil.
[2]Departamento de Ciências Farmacêuticas, Centro de Ciências da Saúde, Universidade Federal da Paraíba, João Pessoa, Paraíba, Brazil.
[3]Departamento de Química Orgânica, Instituto de Química, Universidade Estadual Paulista, Araraquara, São Paulo, Brazil.

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***Arctium lappa* L. (*A. lappa* L) is an important medicinal plant used in Brazil to heal gastric ulcers. This study reveals the pharmacological action of this traditional medicine use. The aqueous extract obtained from the liquid-liquid partition of the 70% ethanol extract of the *A. lappa* was challenged against *in vivo* Hydrochloric acid (HCl)/ethanol-, piroxicam- and cold restrained stress-induced gastric ulcer, and pylorus ligation assays. NO-synthase inhibitor N-nitro-L-arginine methyl ester (L-NAME) and SH blocker (N-ethylmaleimide (NEM) were used to evaluate the participation of cytoprotective factors in ALT gastroprotection. Antiulcerogenic action of *A. lappa* L was evaluated in mice at doses of 50, 100 or 200 mg/kg. *A. lappa* L showed elevated gastroprotective action in all *in vivo* experimental models, but did not interfere with gastric secretion. The mechanisms involved in the gastroprotective action of *A. lappa* L are related to mucosal protector factors, such as nitric oxide (NO) and sulfhydryl (SH) compounds. This species is a promising herbal drug due to its effectiveness in the gastroprotection, which is in accordance with an ethnopharmacological use against gastric ulcers.**

Key words: *Arctium lappa* L, gastric ulcer, gastroprotection.

INTRODUCTION

Herbal preparations have been used for centuries as the most important therapeutic means in some communities worldwide. Plants have formed the basis of sophisticated traditional medicine systems for thousands of years and continue to provide mankind with new remedies (Gurib-Fakim, 2006). Currently, there has been an increasing global interest in non synthetic plant-derived natural medicines due to better tolerance and minimum adverse reactions when compared to synthetic medicines (Musthaba et al., 2010). *Arctium lappa* L. (*A. lappa* L.) (Asteraceae), commonly known as “bardana” is being recommended as a healthy and nutritive food in Chinese communities. It has been therapeutically used in Europe, North America and Asia for hundreds of years (Chan et al., 2010). In Brazil this species is used in folk medicine to treat rheumatism and gastric ulcers (Corrêa, 1984). In addition, it has been reported to posses anti-inflammatory, through the inhibition of pro-inflammatory cytokine expression (Tsai et al., 2011), anti-alergic (Sohn et al., 2011) and anti-ulcerogenic activities (dos Santos et al., 2008). The present work evaluated the anti-ulcerogenic activity of the aqueous fraction obtained from the liquid-liquid partition of the 70% ethanol extract of the leaves of *A. lappa* L. in different models that mime ulcer in human.

*Corresponding author. E-mail: luiz_ferreiraa@yahoo.com.br. Tel: +551935216192. Fax: +5519 35216185.

MATERIALS AND METHODS

Animals

Male Unib: SW mice (30 to 35 g) obtained from the breeding of the State University of Campinas (CEMIB/UNICAMP) Brazil, were housed on a 12 h light/dark cycle at 22±1ºC and 55% humidity, with free access to water and a certified diet (CR-a@, Nuvital, Nuvilab). The mice were fasted for 24 to 36 h prior to all assays because various drugs and aqueous fraction from *A. lappa* L. were administered orally (by gavage – 10 ml/kg) using a saline solution as vehicle. The UNICAMP Institutional Animal Care and Use Committee approved all of the employed protocols following the recommendations of the Canadian Council on Animal Care (Olfert et al., 1993).

Plant material

Leaves of *A. lappa* L. were collected in Mogi Mirim, SP, Brazil and a voucher herbarium specimen (voucher number 131.966) was deposited in the Herbarium of the State University of Campinas.

Fractionation of the 70% ethanol extract

One kilogram of *A. lappa* L. leaves was macerated for one week with 70% ethanol. Rotaevaporation yielded 30 g of hydroalcoholic extract. Liquid-liquid partitioning in a chloroform:water mixture (1:1, v/v) yielded 17 g of chloroform fraction and 13 g of aqueous fraction. The aqueous fraction was utilized in the present study.

Drugs and chemicals

The following drugs were used: cimetidine (Sigma Aldrich, USA), lansoprazole (Medley, Brazil) and piroxicam (Pfeizer, U.S.A.). The chemicals and other solutions used were all of analytical grade. All drugs and reagents were prepared immediately before use.

Antiulcer action

Ethanol/HCl-induced ulcers

The experiments were performed as described by Mizui and Doteuchi (1983). Mice were divided into five groups of six and seven animals each that had fasted 24 h prior to receiving an oral dose of the vehicle, saline (10 ml/kg), lansoprazole (30 mg/kg) or *A. lappa* L (at the doses of 50, 100 or 200 mg/kg body wt.). After 50 min, all groups were orally treated with 0.2 ml of a 0.3-M HCl/60% ethanol solution (HCl/ethanol) for gastric-ulcer induction. Animals were killed 1 h after the administration of HCl/ethanol, and the stomachs excised. The extent of the lesions was measured and the lesion index expressed as the sum of all lesions as described by Szelenyi and Thiemer (1978).

Non-steroidal anti-inflammatory drug-induced gastric ulcers in mice

The experiment was performed according to the method described by Puscas et al. (1997) with modifications. In this model, gastric ulcer was induced using piroxicam (30 mg/kg, s.c.) administered to mice (n = 7) after a 36 h fast. The *A. lappa* L (50 mg/kg), cimetidine (100 mg/kg) or saline was administered orally 30 min before the induction of gastric ulcer. The animals were killed 4 h after treatment with the ulcerogenic agent. The stomachs were removed and ulcerative index calculated as described previously.

Hypothermal-restraint stress ulcers

The hypothermic-restraint stress-induced gastric-ulcer model was assessed in mice by the method of Levine (1971) with modifications. Mice were divided into three groups of five animals each. After 24 h of starvation, the animals received a single oral administration of *A. lappa* L (50 mg/kg), cimetidine (100 mg/kg) or saline. 1 h after treatment, gastric ulceration was induced by immobilizing the animals inside a closed cylindrical cage maintained at 4°C. After 3 h the animals were killed and the stomachs were removed and examined for ulcers as described previously.

Determination of gastric secretion

The assay was performed following the method of Shay et al (1945) with few modifications. All groups of mice (n = 5) fasted for 24 h, with free access to water. Immediately after pylorus ligature, *A. lappa* L (50 mg/kg), cimetidine (100 mg/kg) as positive control, or the vehicle (saline 10 ml/kg) were administered intraduodenally. The animals were killed 4 h later; the abdomen was opened and another ligature placed around the esophagus close to the diaphragm. The stomachs were removed and the gastric content collected to determine the total amount of gastric juice (ml) and pH values. Distilled water was added and the resultant solution centrifuged at 2000 × g for 10 min. Total acid in the gastric secretion was determined in the supernatant volume by titration with 0.05 N NaOH.

Ethanol-induced gastric lesions in L-NAME-pretreated mice

Male mice were divided into three groups of 7 mice each that fasted for 24 h. The animals were pretreated with L-NAME 10 mg/kg or saline i.v., and 30 min later, received an oral dose of saline (10 ml/kg) or *A. lappa* L (50 mg/kg). After 50 min, all groups were treated orally with 1 ml of absolute ethanol for gastric ulcer induction (Sikiric et al., 1997). The animals were sacrificed 1 h after ethanol administration and the stomachs excised and gastric damage determined as described previously.

Ethanol-induced gastric lesions in NEM-pretreated mice

Male mice were divided into three groups of 7 mice each that fasted for 24 h. The animals had been previously treated intraperitoneally with NEM 10 mg/kg or saline, and 30 min later, received an oral dose of saline (10 ml/kg) or *A. lappa* L (50 mg/kg). After 60 min, all groups were treated orally with 1 ml of absolute ethanol to induce gastric ulcer (Arrieta et al., 2003). Animals were sacrificed 1 h after ethanol administration; the stomachs were excised and gastric damage determined as described previously.

Statistical analysis

The results were expressed as the mean ± standard deviation. Statistical comparisons were done by one-way analysis of variance (ANOVA) followed by the Dunnett's or Tukey's post-hoc test, with the level of significance set at $p < 0.05$.

RESULTS AND DISCUSSION

Peptic ulcers are a common disorder of the entire gastrointestinal tract that occurs mainly in the stomach and the proximal duodenum (Mota et al., 2009). The

Table 1. Effects of ALT from *A. lappa* on different models of acute gastric lesion induced in mice.

Gastric lesions model	Treatment (p.o.)	Dose (mg/kg)	ULI (mm)	Inhibition (%)
Ethanol/HCl	Saline	10 ml/kg	28.0 ± 6.7	-
	Lansoprazole	30	6.8 ± 1.5***	75
	ALT – *Arctium lappa*	50	10.0 ± 3.4***	64
		100	15.0 ± 2.4***	46
		200	8.4 ± 4.3***	70
Piroxicam	Saline	10 ml/kg	11.0 ± 0.8	-
	Cimetidine	100	5.0 ± 3.2**	54
	ALT – *Arctium lappa*	50	4.8 ± 3.3**	56
Stress	Saline	10 ml/kg	12.2 ± 4.2	-
	Cimetidine	100	3.9 ± 2.8***	68
	ALT – *Arctium lappa*	50	5.9 ± 3.0**	51

The results are the mean ± S.D. ANOVA followed by Tukey's test. ** $p<0.01$, *** $p < 0.001$ compared to the corresponding saline group.

various animal models for peptic ulcers have played a significant role in the elucidation of the peptic ulceration (Lee, 2000). Since it is a multifactorial disease, its treatment faces great difficulties due to the limited effectiveness and severe side effects of the currently available drugs (Mota et al., 2009). In this context, numerous plant-derived natural products have been evaluated as therapeutics for the treatment of a variety of diseases, including the peptic ulcer (de Sousa Falcão et al., 2008; Musthaba et al., 2010). Ethanol is one of the ulcerogenic agents that induce intense damage in gastric mucosa by promoting disturbances in the mucosal microcirculation, ischemia and appearance of free radicals, endothelin release, degranulation of mast cells, inhibition of prostaglandins and decrease in gastric mucus production (Abdel-Salam et al., 2001). Oral administration of HCl/ethanol produced multiple band-like lesions in the glandular mucosa, along the long axis of the stomach (Takeuchi et al., 2010). The oral administration of ethanol/HCl to the control group produced the characteristic zonal necrotic mucosal lesions.

The aqueous fraction from the liquid-liquid partition of the 70% ethanol extract of the leaves of *A. lappa* L. (50, 100 and 200 mg/kg) and lanzoprazole (30 mg/kg, positive control) significantly inhibited ulcer formation in this model by 75, 64, 46 and 70%, respectively (Table 1). The reduction of the lesions seen with *A. lappa* L. suggested that part of the protective mechanism could involve mucosal defensive factors. Gastric lesions caused by necrotizing agents are not inhibited by anti-secretory agents like cimetidine, but by mucosal defensive factor enhancers (Morimoto et al., 1991). In the first model (HCl-induced gastric lesions), all the doses were effective however, we chose the minor one (50 mg/Kg) to continue our studies in subsequent assays. The nonselective Nonsteroidal anti-inflammatory drugs (NSAIDs) induce predictable gastric mucosal injury either by intimate contact with the mucosa or by the inhibition of cyclooxygenase, diminishing the production of Prostaglandins (PGs), which plays a pivotal role in the gastric protection (Laine et al., 2008). PGs regulate the secretion of mucin and surface active phospholipids by mucous cells (Takeuchi et al., 2010), and aspirin and other NSAIDs significantly inhibit basal bicarbonate secretion from gastric and duodenal mucosa. *A. lappa* L (50 mg/kg) significantly inhibited ulcer formation in the piroxicam-induced ulcer model (Table 1).

This result confirmed our hypothesis that a cytoprotective mechanism was involved with the antiulcerogenic effect of *A. lappa* L. Physical and psychological stresses are widely accepted as triggers and/or modifiers of the clinical course of diverse gastrointestinal disorders such as peptic ulcer (Caso et al., 2008). Stress can also synergize with other pathogenic factors such as *Helicobacter pylori* infection and NSAIDs to produce gastrointestinal diseases (Caso et al., 2008). Pre-treatment with *A. lappa* L (50 mg/kg) significantly protected the gastric mucosa against ulcers in this model when compared with the control (Table 1). This finding suggested that *A. lappa* L was able to enhance some protective factors of gastric mucosa. It is known that PGs is effective in preventing the occurrence of gastric lesions under stress conditions such as cold-restraint, mainly mediated by prostacyclin (Takahira et al., 2007; Takeuchi et al., 2010). Aiming at the investigation of the participation of *A. lappa* L (50 mg/kg) in the gastric acid secretion, we have evaluated the biochemical parameters of gastric juice in the pylorus-ligated model. Cimetidine (100 mg/kg) strongly inhibited gastric acid secretion as shown by a decrease in the total acid content of gastric juice.

Table 2. Effects of ALT (50 mg/kg) from *A. lappa* administered by intraduodenal route on biochemical parameters of gastric juice obtained from pylorus ligature mice.

Treatment	Dose (mg/kg)	pH (units)	Volume (mL)	Total acid productuin (mEq/4h/mL)
Saline	mL/kg	2.9 ± 0.9	0.36 ± 0.37	5.58 ± 2.49
Cimetidine	100	4.0 ± 0.5*	0.15 ± 0.07	11.7 ± 7.76*
ALT	50	3.5 ± 1.3	0.29 ± 0.17	8.25 ± 5.04

The results are the mean ± S.D. ANOVA followed by Dunnett's test.*, $p < 0.05$ compared to the saline group.

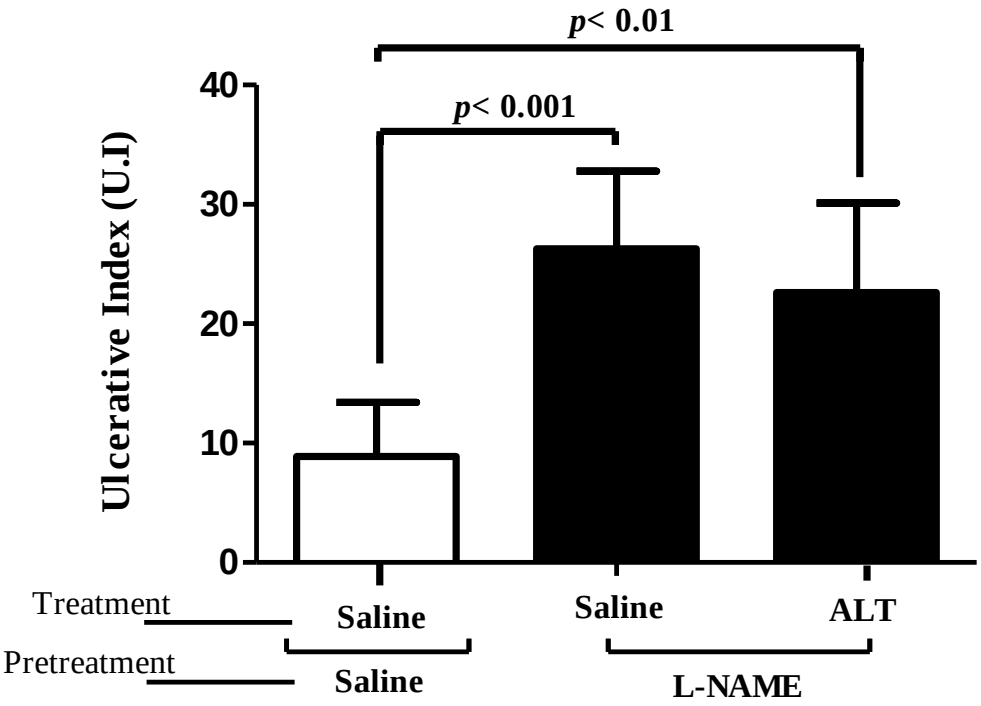

Figure 1. The ulcer index for gastric ulcers induced by HCl/ethanol in mice pretreated with L-NAME (10 mg/kg) alone and ALT (50 mg/kg) of *A. lappa*. The results are reported as the mean ± S.D. ANOVA followed by Tukey's test.

In contrast, *A. lappa* L did not alter the biochemical parameters of the gastric juice (Table 2). This finding indicates that the protective action of *A. lappa* L was independent from acid production. Besides PGs, NO also modulates many of the same elements of mucosal defense (blood flow, mucus and bicarbonate secretion) (Wallace, 2008). Pretreatment with L-NAME, an NO sintase blocker, aggravated the ethanol-induced mucosal lesions. The length and the score of the lesions from *A. lappa* L (50 mg/kg) groups were higher (Figure 1). This finding indicates that NO may be involved in the protective action of *A. lappa* L. The improved blood flow associated with increased NO delivery was an important factor in countering the harmful effects of HCl, ethanol and NSAID (Kitagawa et al., 1990; Masuda et al., 1995; Wallace et al., 1994).

The mucous secretion constitutes the first line of mucosal defense against haptenating agents playing an important role in gastric protection. SH protect the mucus maintaining the disulfide bridges. If these bridges are reduced, the mucus becomes more soluble and more susceptible to the haptenating agents. Accordingly, the SH are the key agents in mucosal protection against ethanol-induced gastric injury (Avila et al., 1996). Ethanol is known to deplete SH and promote lipid peroxidation and pretreatment with sulfhydryl blockers prevented the gastroprotection of sulfhydryl-containing substances (Szabo and Brown, 1987). Our findings corroborated with earlier reports showing depletion of SH compounds in ethanol-induced gastric lesions (Luiz-Ferreira et al., 2010). Pretreatment with NEM, a sulfhydryl blocker, abrogated the protection afforded by *A. lappa* L (50 mg/kg) (Figure 2), which suggested that endogenous sulfhydryls may be involved in the protective mechanism

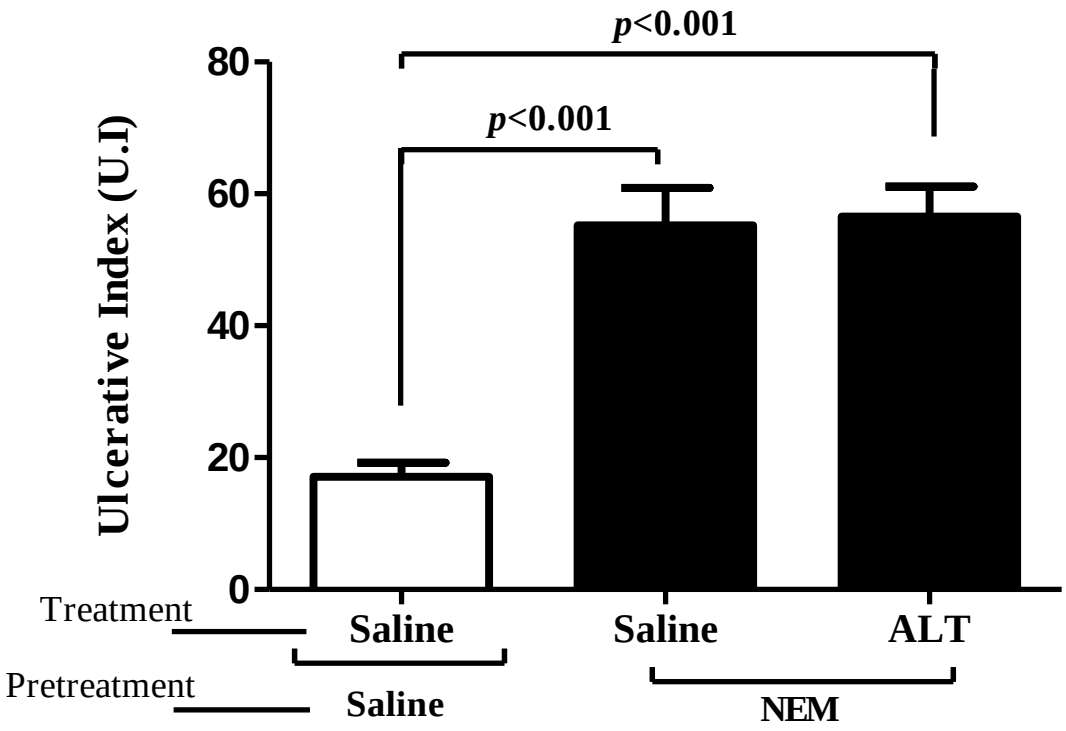

Figure 2. The ulcer index for gastric ulcers induced by ethanol in rats pretreated with NEM (10 mg/kg) alone and ALT (50 mg/kg) of *A. lappa*. The results are reported as the mean ± S.D. ANOVA followed by Tukey's test.

of *A. lappa* L.

Conclusions

The data obtained the aqueous fraction from the liquid-liquid partition of the 70% ethanol extract of the leaves of *A. lappa* L. confirmed the traditional medicinal uses for this species. The gastroprotective mechanism is dependent on defensive factors of the mucosa, such as endogenous sulfhydryls and NO. Further studies are required to confirm the others mechanism underlining the protecting property of the extract and to identify the chemical constituents responsible for it.

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