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Brazilian Medicinal Plant Acts on Prostaglandin Level and *Helicobacter pylori*

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ABSTRACT Among the current treatment strategies for the peptic ulcer patient with *Helicobacter pylori* infection, the method of choice is triple therapy based on the concurrent use of proton inhibitors and two antibiotics. *Alchornea triplinervia* is a medicinal plant commonly used by people living in the Cerrado region of Brazil to treat gastrointestinal ulcers. In the present work we proposed therapy based on this medicinal plant that presents effective gastroprotective action with antibiotic effects. Oral pretreatment with methanolic extract (ME) of *A. triplinervia* in rats and mice decreased the gastric injuries induced by ethanol and HCl/ethanol. Increasing the dose reduced the gastroprotective effects of ME on the gastric lesions induced by nonsteroidal anti-inflammatory drug. After pylorus ligature of mice, oral administration of ME induced a decrease not only in total acid but also in the ulcer index. We also observed that ME displayed antibacterial activity against *H. pylori*. Liquid-liquid separation of ME indicated that active constituents responsible for the gastroprotective action are concentrated in the ethyl acetate fraction (EAF) (50% protection) rather than in the aqueous fraction, which did not induce significant gastroprotection at the same dose (100 mg/kg). EAF induced an increase of gastric mucosa prostaglandin (PG) E_2 levels, which remained high even after previous administration of indomethacin. The phytochemical profile of ME revealed that EAF contains mainly flavonoids. In conclusion, all these results suggest that ME did not show acute toxicity, but exhibited an antisecretory property, anti-*H. pylori* effect, and gastroprotective action. The observed effect did not involve the participation of nitric oxide or endogenous sulfhydryl groups. However, EAF showed a more efficient gastroprotective effect than ME at a lower dose and protected the gastric mucosa by increasing PGE_2.

KEY WORDS: • **Alchornea triplinervia** • ***flavonoids*** • ***gastroprotective action*** • **Helicobacter pylori** • ***prostaglandin E_2***

INTRODUCTION

PEPTIC ACID ULCERS and diseases have been on the rise in today's era of globalization, which is characterized by hurry, worry, and curry.[1] This disease is attributed to the imbalance between aggressive factors (including acid, pepsin, and *Helicobacter pylori* infection) and local mucosal defenses (like secretion of bicarbonate, mucus, and prostaglandins [PGs]).[2] The predominant causes of peptic ulcer disease in the United States are infection with *H. pylori* and use of nonsteroidal anti-inflammatory drugs (NSAIDs). *H. pylori* can be eradicated by triple therapy consisting of two antimicrobial agents and a proton pump inhibitor, such as lansoprazole. However, these drugs are mainly influenced by bacterial susceptibility and resistance to antimicrobial agents as well as the magnitude of acid inhibition during the treatment. Patients taking NSAIDs should discontinue their use because of peptic ulcers.[3]

Despite the progress through conventional chemistry and pharmacology in producing effective drugs, the plant kingdom may provide useful sources of new anti-ulcer compounds for development as pharmaceutical entities or, alternatively, as simple dietary adjuncts to existing therapies.[4]

The leaves and aerial parts of *Alchornea triplinervia* are commonly used in Brazilian folk medicine in tea form to treat gastric disturbances.[5] However, there are no pharmacological or toxicological studies on this species. The only report about *A. triplinervia* describes the isolation of amentoflavone, isocorilagine, gallic acid, and methyl gallate from leaves.[6] In contrast, other species of this genus already have been subjects of pharmacological studies: *Alchornea cordifolia* presented an anti-inflammatory property, whereas *Alchornea castaneaefolia* and *Alchornea glandulosa* exhibited an anti-ulcer action.[7–9] Ebi[10] determined that methano-

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lic extract (ME) of *A. cordifolia* also showed antimicrobial activity against *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Escherichia coli*.

The present study was carried out to investigate the gastroprotective and anti-*H. pylori* effects of ME from *A. triplinervia*, as well as to evaluate the possible mechanism of action.

MATERIALS AND METHODS

Drugs and chemicals

The chemicals and other solutions used were all of analytical grade. All drugs and reagents were prepared immediately before use. The following drugs were used: *N*-nitro-L-arginine methyl ester (L-NAME), cimetidine, *N*-ethylmaleimide (NEM), indomethacin, and carbenoxolone from Sigma Chemical Co. (St. Louis, MO) and lansoprazole and piroxicam from Pfizer (São Paulo, Brazil). The extract and fractions were dissolved in saline (0.9% NaCl).

Plant material and preparation of extract and fractions

Leaves of *A. triplinervia* were collected at Botucatu, São Paulo State, Brazil, in August 2003, and the vegetal species was identified by Prof. Dr. Jorge Tamashiro from Campinas State University, Campinas, SP, Brazil. A voucher specimen (BOTU number 14873) was deposited at the Herbarium of the São Paulo State University campus in Botucatu, SP, Brazil. The leaves (500 g) of *A. triplinervia* were air-dried (7 days at 40°C) and powdered. The powdered aerial parts were exhaustively extracted with methanol successively at room temperature (three times, 72 hours for each solvent) to produce the ME with a yield of 15% (75 g). A portion (28 g) of the ME was separated into ethyl acetate/water (1:1, vol/vol), leading to 7.5 g (27%) of the ethyl acetate fraction (EAF) and 15 g (54%) of the aqueous fraction (AqF). Both layers were checked by thin-layer chromatography (silica gel plates, chloroform/methanol/*n*-propanol/water [5:6:1:4 by volume], organic phase) and visualized with anisaldehyde sulfuric acid reagent.[11] Flavonoids (yellow spots) and gallic acid derivatives (gray spots) were concentrated in the EAF, whereas tannins and free sugars remained in the aqueous layer.

Analytical and quantitative measurement of total phenolic compounds by high-performance liquid chromatography–ultraviolet (UV)-photodiode array

In the AqF and EAF, the flavonoid concentration was determined as follows. An aliquot of each fraction (30 mg) was dissolved in 3 mL of water/methanol (8:2, vol/vol), filtered in a Sep-Pak cartridge (C_{18}, 500 mg; Sigma), and analyzed using a Varian (Walnut Creek, CA) ProStar high-performance liquid chromatography system equipped with an RP-18 column (250 × 4.60 mm i.d., 5 μm, Luna; Phenomenex, Torrance, CA) and a Rheodyne (Cotati, CA) model 7125 sample injector with a 20-μL sample loop. The mobile phase was water (A) and acetonitrile (B), both with 0.05% trifluoroacetic acid, in linear gradient elution of 30–70% of B for 60 minutes at a flow rate of 1.0 mL/minute. The effluent was monitored using a ProStar 330 photodiode array UV detection system at 360 nm. Compounds were identified by retention time and characteristic UV spectra and by spiking with standards and isolated compounds from a collection in our laboratory under the same conditions: ellagic acid (**1**), quercetin-3-*O*-galactopyranoside (**2**), quercetin-7-*O*-glucopyranoside (**3**), quercetin-3-*O*-glucopyranoside (**4**), and quercetin-3-*O*-arabinopyranoside (**5**). A stock solution (1 mg/mL) of rutin was prepared in methanol. The calibration curve was constructed utilizing seven different concentrations (10, 20, 50, 100, 200, 300, and 500 μg/mL in rutin) and analyzed in triplicate. The peak areas were correlated with the concentrations according to the calibration curve. A calibration curve using the external standard rutin was constructed to determine the total concentration of flavonoids. The calibration curve was linear over the range of 10–500 μg/mL with a correlation coefficient of 0.9999. All data are presented as mean ± standard deviation of four independent experiments ($n = 4$).

Animals

Male Swiss albino mice (weighing 25–35 g) and male Wistar albino rats (weighing 150–250 g) from the São Paulo State University Central Animal House were used. The animals were fed a certified Nuvilab (Nuvital Nutrientes, Colombo, Brazil) diet with free access to tap water under standard conditions of 12 hours dark/12 hours light, humidity (60 ± 1.0%), and temperature (21 ± 1°C). Fasting was used prior to all assays because standard drugs were always administered orally (by gavage) or intraduodenally. Saline solution (10 mL/kg) was always used as the vehicle. Moreover, the animals were kept in cages with raised floors of wide mesh to prevent coprophagy. All experiments were performed in the morning and followed the recommendations of the Canadian Council on Animal Care.[12] The São Paulo State University Institutional Animal Care and Use Committee approved all of the protocols used.

Acute toxicity

The acute toxicity studies were performed in male and female mice ($n = 10$). A single dose of ME was administered orally to groups of animals after a 12-hour fast. Animals receiving saline served as controls. The signs and symptoms associated with ME administration (5 g/kg, p.o.) were observed at 0, 30, 60, 120, 180, and 240 minutes after and then once a day for the next 14 days. At the end of the ME treatment period the number of survivors was recorded. The acute toxicological effect was estimated by the method described by Souza Brito.[13]

Gastroprotective activity

HCl/ethanol-induced ulcer. The experiment was performed as described by Mizui and Doteuchi.[14] Mice were divided into groups of six or seven animals each that had undergone fasting 24 hours prior to receiving an oral dose of the vehicle (saline), lansoprazole (30 mg/kg), or ME (at a dose of 250, 500, or 1,000 mg/kg of body weight). After 50 minutes, all groups were treated orally with 0.2 mL of a 0.3 *M* HCl/60% ethanol solution (HCl/ethanol) to induce gastric ulcer. Animals were sacrificed 1 hour after the administration of ethanol solution, and stomachs were excised. The extent of the lesions was measured, and the lesion index was expressed as the sum of all lesions.[15]

Ethanol-induced ulcer. Rats were divided into groups of five to seven animals each that had undergone fasting 24 hours prior to receiving an oral dose of the vehicle, lansoprazole (30 mg/kg), ME (250, 500, or 1,000 mg/kg), EAF (100 mg/kg), or AqF (100 mg/kg). After 60 minutes, all groups were treated orally with 1 mL of absolute ethanol to induce gastric ulcer.[16] Animals were sacrificed 1 hour after ethanol administration, and stomachs were excised and gastric damage was determined as described above.

NSAID gastric ulcers in mice. In this model, mice were divided into groups of seven animals each, and gastric lesions were induced with piroxicam (30 mg/kg, s.c.).[17] ME (250, 500, or 1,000 mg/kg), cimetidine (100 mg/kg), or saline was administered orally 30 minutes before the induction of gastric lesion by NSAID. The animals were sacrificed 4 hours after treatment, and stomachs were removed and gastric damage was determined as described above.

Shay et al.*[18] ulcer production method*. Mice were randomly divided into groups of seven to nine animals each that had undergone fasting for 24 hours with free access to water. Thirty minutes after oral dosing or immediately after intraduodenal administration of a single dose of ME (500 mg/kg), cimetidine (100 mg/kg) as the positive control, or vehicle (saline), pylorus ligature was performed.[18] Four hours later the animals were sacrificed, the abdomen was opened, and another ligature was placed around the esophagus close to the diaphragm. The stomach was removed and inspected internally, and its contents were drained into a graduated centrifuge tube and centrifuged at 3,500 *g* for 15 minutes. The supernatant volume and pH were recorded with a digital pH meter (PA 200, Marconi SA, Campinas). The total acid content of gastric secretion was also determined by titration to pH 7.0 with 0.01 *N* NaOH using a digital burette (E.M., Hirschmann Technicolor, Eberstadt, Germany).

Ethanol-induced gastric lesions in L-NAME-pretreated rats. The rats were divided into six groups of seven rats each that had undergone fasting for 24 hours. The animals were treated with L-NAME (70 mg/kg) or saline intraperitoneally and 30 minutes later received an oral dose of the vehicle (10 mL/kg), ME (500 mg/kg), or carbenoxolone (100 mg/kg). After 60 minutes, all groups were treated orally with 1 mL of absolute ethanol to induce gastric ulcers.[19] Animals were sacrificed 1 hour after ethanol administration, and the stomachs were excised and gastric damage was determined as described above.

Ethanol-induced gastric lesions in NEM-pretreated rats. Rats were divided into six groups of seven rats each that had undergone fasting for 24 hours. The animals had been previously treated intraperitoneally with NEM (10 mg/kg) or saline and 30 minutes later received an oral dose of the vehicle (10 mL/kg), ME (500 mg/kg), or carbenoxolone (100 mg/kg). After 60 minutes, all groups were treated orally with 1 mL of absolute ethanol to induce gastric ulcers.[19] Animals were sacrificed 1 hour after ethanol administration, and the stomachs were excised and gastric damage was determined as described above.

PG synthesis determination. Rats were divided into three groups of six animals each that, after a 24-hour fast, received one of the following solutions: saline (vehicle), EAF (100 mg/kg), or the combination of EAF (100 mg/kg) plus indomethacin (20 mg/kg, s.c). In this last group, EAF was administered, followed 30 minutes later by indomethacin (dissolved in 5% sodium bicarbonate solution). Thirty minutes after treatments, all the animals were sacrificed, and the abdomen was opened. A sample of the corpus (full thickness) was excised, weighed, and suspended in 1 mL of 1 m*M* sodium phosphate buffer, pH 7.4. The tissue was finely minced with scissors and then incubated at 37°C for 20 minutes. PGE_2 in the buffer was measured by enzyme immunoassay (Amersham Biosciences, Little Chalfont, Buckinghamshire, UK), and the absorbance was read at 450 nm.[20]

*Anti-*H. pylori *activity*

The ME was tested to detect anti-*H. pylori* activity.[21] The strain of *H. pylori* (ATCC 43504) had been isolated from patients with duodenal ulcer disease. The frozen *H. pylori* isolate was thawed and grown on 5% sheep blood agar plates for 3–4 days at 37°C in 10% CO_2 and 98% humidity. Each plate was swabbed with a sterile cotton-tipped applicator, and the cells were suspended in sterile saline to obtain turbidity equivalent to a 2.0 McFarland standard. Mueller-Hinton broth containing 10% horse serum was added to all wells of a 96-well microtiter plate (Corning, Corning, NY). Each well was incubated with *H. pylori* at a final concentration of 1×10^5 colony-forming units/mL. The plates were incubated for 5 days in a microaerobic atmosphere at 37°C. Following incubation, the plates were examined visually and spectrophotometrically, and the lowest concentration showing complete inhibition of growth was recorded as the minimum inhibitory concentration (MIC). *Staphylococcus aureus* ATCC 25923 and *E. coli* ATCC 25922 were used as control organisms for clarithromycin and ampicillin, respectively. The results were considered valid only when the

MIC values for the control organisms were within the ranges established by the National Committee for Clinical Laboratory Standards (Wayne, PA).

Statistical analysis

Results were expressed as mean ± SE values, and statistical significance was determined by one-way analysis of variance followed by Dunnett's or Tukey's test with $P < .05$ defined as significant.

RESULTS AND DISCUSSION

The balance between the therapeutic versus the toxicological effects of a drug is an important parameter in assessing its applicability as an anti-ulcer or a pharmacological agent.[22] As a part of this pharmacological study, ME was first investigated for acute toxicity in mice. A single oral dose of ME (5 g/kg) did not produce any visible signs or symptoms of toxicity in the treated animals. After 14 days of administration, no animal died, and no significant macroscopic changes in daily body or organ weights were observed (data not shown). Since no acute toxicity was observed using ME, we continued our studies evaluating the effect of ME administered to rodents using different standard experimental models of induced gastric ulcer.

According to Souza Brito[13] active principles from medicinal plants are generally present at low concentrations. So, in order to establish a general profile of the anti-ulcerogenic activity of the extract, we plotted the dose–response curve using three peroral doses of 250, 500, and 1,000 mg/kg to select the dose that produces the best effect.

Oral administration of absolute ethanol is noxious to the stomach by affecting the gastric mucosa topically by disrupting its barrier and provoking pronounced microvascular changes within a few minutes after its application. Konturek *et al*.[23] reported that the combination of HCl plus ethanol also promotes stasis in gastric blood flow that contributes to the development of the hemorrhagic and necrotic aspects of tissue injury. Our results obtained with absolute ethanol and HCl/ethanol models gave similar results with 85–86% and 89–90% gastroprotection at the respective ME doses of 500 and 1,000 mg/kg (Table 1). These data suggest that ME displays a gastroprotective effect since it significantly reduced the occurrence of ethanol-induced ulcers.

It is commonly postulated that a mechanism that causes gastroduodenal damage involves cyclooxygenase-1 inhibition that results in gastric PG suppression.[24] Deficiency of endogenous PGs is widely accepted as a major factor in the pathogenesis of gastric lesions caused by NSAIDs.[25,26] However, our data showed that in the NSAID-induced gastric lesion model, the ME-treated animals exhibited an un-

Table 1. Gastroprotective Effects of ME, EAF, or AqF from *A. triplinervia* on Gastric Lesions Induced by HCl/Ethanol, Piroxicam, and Absolute Ethanol in Rodents

Model (animal)	*Treatment (dose)*	*Number*	*ULI*	*Inhibition (%)*
HCl/ethanol (mice)[a]	Vehicle	7	104.0 ± 6.3	—
	Lansoprazole	7	18.0 ± 1.9**	83
	ME (250 mg/kg)	7	23.2 ± 2.1**	78
	ME (500 mg/kg)	6	14.8 ± 1.3**	86
	ME (1,000 mg/kg)	6	10.8 ± 1.8**	90
Piroxicam (mice)[b]	Vehicle	7	32.8 ± 3.3	—
	Cimetidine	7	6.7 ± 1.2**	80
	ME (250 mg/kg)	7	23.4 ± 3.6	—
	ME (500 mg/kg)	7	41.0 ± 4.55	—
	ME (1,000 mg/kg)	7	58.0 ± 4*	−77
Ethanol (rats)				
ME treatment[c]	Vehicle	7	65.8 ± 6.7	—
	Lansoprazole	7	29.0 ± 4.2*	56
	ME (250 mg/kg)	7	47.7 ± 16.6	—
	ME (500 mg/kg)	7	10.1 ± 1.3**	85
	ME (1,000 mg/kg)	7	7.0 ± 1.4**	89
EAF treatment[d]	Vehicle	5	100.7 ± 11.9	—
	Lansoprazole	6	21.3 ± 5.6**	79
	EAF (100 mg/kg)	6	50.5 ± 2.5**	50
AqF treatment[d]	Vehicle	6	92.8 ± 16.2	—
	Lansoprazole	6	31.3 ± 5.0**	66
	AqF (100 mg/kg)	6	71.8 ± 10.1	—

Data are mean ± SE values. ULI, ulcerative lesion index.
For statistical analysis, analysis of variance followed by Dunnett's test was employed: *$P < .05$, **$P < .01$.
[a]$F_{4;\,29} = 138.5$.
[b]$F_{4;\,30} = 27.2$.
[c]$F_{4;\,30} = 28.6$.
[d]$F_{5;\,29} = 47.47$.

TABLE 2. EFFECTS OF ME OF *A. TRIPLINERVIA* ADMINISTERED ORALLY OR INTRADUODENALLY ON GASTRIC JUICE PARAMETERS IN PYLORUS LIGATURE-INDUCED GASTRIC LESIONS IN MICE

Route, treatment	*Number*	*Dose (mg/kg)*	*Total acid (μEq/mL/ 4 hours)*	*pH (unit)*	*Gastric volume (mL)*	*ULI*	*Inhibition (%)*
Intraduodenal							
Control	9	—	32.11 ± 1.18	2.50 ± 0.34	0.98 ± 0.07	49.72 ± 4.86	—
Cimetidine	8	100	18.77 ± 2.22**	3.90 ± 0.43*	0.82 ± 0.06	12.50 ± 1.95**	75
ME	8	500	29.72 ± 1.98	1.70 ± 0.26	1.12 ± 0.03	19.80 ± 1.63**	60
Peroral							
Control	7	—	46.57 ± 3.50	3.00 ± 0.26	0.90 ± 0.09	46.57 ± 3.52	—
Cimetidine	8	100	16.87 ± 2.25**	2.50 ± 0.19	1.09 ± 0.08	22.14 ± 3.32**	52
ME	8	500	4.00 ± 0.50**	3.30 ± 0.37	1.05 ± 0.11	9.25 ± 1.16**	80

Data are mean ± SE values. ULI, ulcerative lesion index.

For statistical analysis, analysis of variance followed by Dunnett's test was employed. By the intraduodenal route: $^{**}P < .01$ for total acid with $F_{2;\,22} = 15.37$, $^{*}P < .05$ for pH with $F_{2;\,22} = 9.99$; $P > .05$ for gastric volume with $F_{2;\,22} = 13.95$, and $^{**}P < .01$ for ULI with $F_{2;\,22} = 39.01$. By the peroral route: $^{**}P < .01$ for total acid with $F_{2;\,20} = 93.11$, $^{*}P < .05$ for pH with $F_{2;\,20} = 2.03$, $P > .05$ for gastric volume with $F_{2;\,20} = 0.94$, and $^{**}P < .01$ for ULI $F_{2;\,20} = 47.47$.

expected result, *i.e.*, the cytoprotection decreased as the dose was increased (Table 1). In this model, ME showed an absence of gastroprotective effect ($P > .05$) at doses of 250 mg/kg and 500 mg/kg, while the dose of 1,000 mg/kg induced a significant increase of gastric lesions ($P < .05$). It is highly probable that ME contains different active constituent(s), *i.e.*, the substance or substances that protected the gastric mucosa against the damage induced by absolute ethanol and HCl/ethanol are different from those that presented cytoprotection against the NSAID-induced gastric lesions. Among natural substances of plant origin, the literature reports that flavonoids may present this type of activity. Gracioso *et al.*[27] reported that increasing the dose of flavonoids changes their antioxidant activity to a pro-oxidant action, leading to an increase in gastric damage. The literature indicates that other species of the *Alchornea* genera present anti-inflammatory activity.[7] Our results agree closely with those published by Repetto and Llesuy,[28] which showed that phenolic compounds have a dual effect on PG biosynthesis, since low concentrations stimulate whereas high concentrations inhibit PGH synthase. Thus, the action mechanism possibly involves the modulation of endogenous PGs by active constituents contained in ME.

With the purpose of investigating the probable gastroprotective mechanisms involved in the action promoted by this extract, we continued our studies using only a single

TABLE 3. EFFECTS OF ME OF *A. TRIPLINERVIA* ON GASTRIC LESIONS INDUCED BY ETHANOL IN RATS PRETREATED WITH NEM OR L-NAME ($N = 7$)

Pretreatment (intraperitoneal)	*Treatment (peroral)*	*Dose (mg/kg)*	*ULI (% inhibition)*
Saline	Vehicle	—	73.7 ± 6.7[a]
	Carbenoxolone	100	31.8 ± 5.9 (57%)**
	ME	500	26.6 ± 2.8 (64%)**[b]
NEM (10 mg/kg)	Vehicle	—	148.3 ± 15.3[a]
	Carbenoxolone	100	106.3 ± 16.3 (28%)*
	ME	500	40.6 ± 4.5 (73%)**[b]
Saline	Vehicle	—	135.5 ± 18.8[c]
	Carbenoxolone	100	24.0 ± 5.0 (82%)**
	ME	500	20.1 ± 14.9 (85%)**[d]
L-NAME (70 mg/kg)	Vehicle	—	197.7 ± 15.0[c]
	Carbenoxolone	100	81.7 ± 20.4 (59%)*
	ME	500	41.0 ± 8.5 (79%)**[d]

Data are mean ± SE values. ULI, ulcerative lesion index.

Analysis of variance followed by Dunnett's test was used to determine significant differences from the respective control group: $^{*}P < .05$, $^{**}P < .01$.

[a]Saline/vehicle versus NEM/vehicle ($P < .05$).

[b]Saline/ME versus NEM/ME ($P > .05$) by analysis of variance followed by Tukey's test.

[c]Saline/vehicle versus L-NAME/vehicle ($P < .05$).

[d]Saline/ME versus L-NAME/ME ($P > .05$) by analysis of variance followed by Tukey's test.

dose of ME (500 mg/kg) in subsequent assays, since this had produced the best results in the previous studies, and no significant differences were observed between groups treated with 500 or 1,000 mg/kg ME.

In pyloric ligation, the digestive effect of accumulated gastric juice (gastric hypersecretion) and the interference of gastric blood circulation are responsible for the induction of ulceration.[29,30] Animals pretreated orally with ME presented a decrease of ulcerative lesions and a drop in the total acid value in gastric juice (Table 2). Otherwise, the intraduodenal administration of ME did not change any gastric juice biochemical parameter but decreased the ulcerative index. Therefore, these data indicate that ME presented antisecretory action only when this extract was given orally and that intestinal absorption of ME did not contribute to its antisecretory effect.

Ethanol-induced gastric damage is associated with a significant decrease in the mucosal sulfhydryl (SH) level, including reduced gluthathione, and pretreatment with SH blockers prevents gastroprotection of SH-containing compounds.[31,32] Our data show that pretreatment with the SH blocker NEM did not reduce the mucosal protection observed with ME treatment. These findings suggest that an increase of endogenous SH is not involved in the gastroprotective effect of ME, as shown in Table 3.

Vascular changes in gastric mucosa appear to be the most pronounced feature of absolute ethanol-induced injury.[33] Maintenance of mucosal vasculature and normal blood flow may constitute the major cytoprotective mechanism.[34] As shown in Table 3, L-NAME, a nitric oxide synthase inhibitor, also did not attenuate the gastroprotection observed for ME, suggesting that endogenous nitric oxide did not participate in the protective effect of ME.

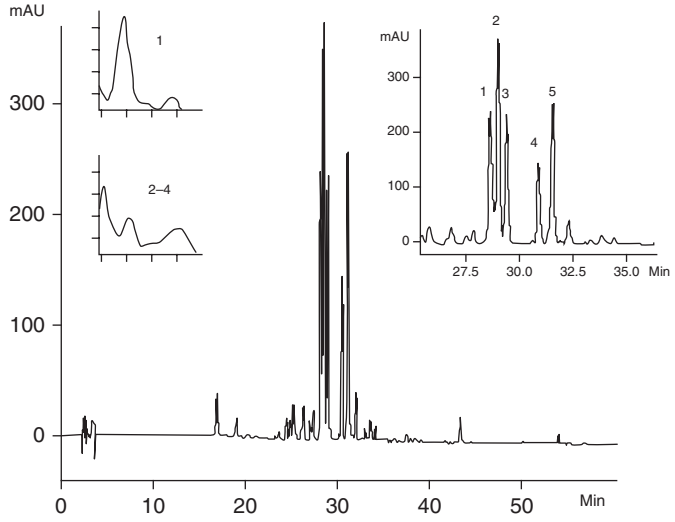

FIG. 1. High-performance liquid chromatography profile of EAF from *A. triplinervia*. Chromatographic conditions were as follows: RP-18 column, 250 × 4.6 mm (i.d.), 5 μm; elution with water (0.05% trifluoroacetic acid)/acetonitrile (0.05% trifluoroacetic acid), 30% to 70% in 60 minutes; flow rate, 1.0 mL/minute; λ = 360 nm. mAU, milli-absorbance units.

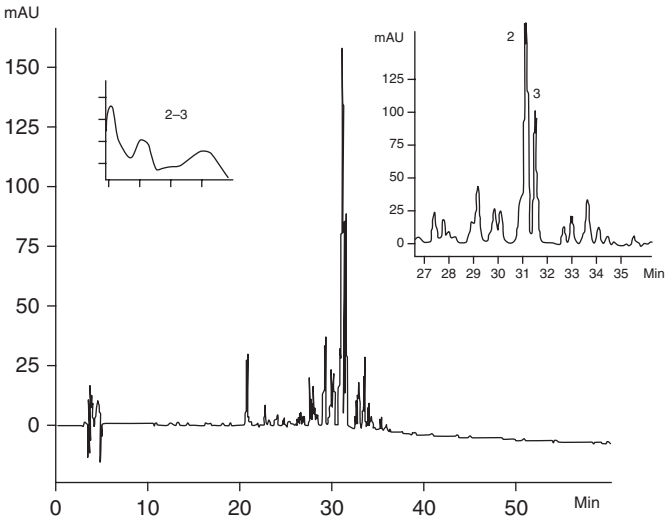

FIG. 2. High-performance liquid chromatography profile of AqF from *A. triplinervia*. Chromatographic conditions were as follows: RP-18 column, 250 × 4.6 mm (i.d.), 5 μm; elution with water (0.05% trifluoroacetic acid)/acetonitrile (0.05% trifluoroacetic acid), 30% to 70% in 60 minutes; flow rate, 1.0 mL/minute; λ = 360 nm. mAU, milli-absorbance units.

We also evaluated the anti-*H. pylori* activity of ME. In the course of the study, *A. triplinervia* ME was found to present antibacterial activity against the standard strain of *H. pylori* (ATCC 43504). The results showed that the MIC of ME against *H. pylori* was 0.25 mg/mL. The literature reported that an MIC of <0.50 mg/mL is considered interesting for extracts.[21,35] We conclude that ME presents excellent antimicrobial action against one of the most important factors that cause gastric ulceration.

In order to better comprehend the effect of ME on gastric injuries, we separated ME into ethyl acetate and water, thus obtaining two fractions (EAF and AqF). The pretreatments with these fractions were also evaluated against the ethanol-induced gastric lesions that had resulted in the best results with ME. We observed that the active constituents responsible for the protective action are concentrated in the EAF (50% protection), rather than in the AqF, which did not induce significant gastric protection at the same dose (Table 1). Therefore, we can conclude that the active constituents responsible for the protective action are concentrated in the EAF and not in the AqF.

We also determined spectrophotometrically the total concentration of flavonoids in the *A. triplinervia* leaves. The flavonoid contents were 19.73% (197.3 ± 0.19 mg/g) in the EAF and 1.3% (13.1 ± 0.04 mg/g) in the AqF from ME. In agreement with these results, Figures 1 and 2 present the chromatograms and UV spectra of the respective fractions EAF and AqF from ME. The EAF contained primarily five phenolic compounds: ellagic acid, quercetin-3-*O*-galactopyranoside, quercetin-7-*O*-glucopyranoside, quercetin-3-*O*-

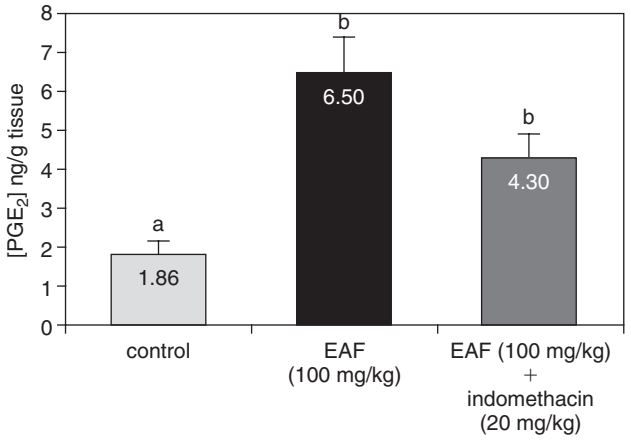

FIG. 3. Effect of EAF from *A. triplinervia* on PGE_2 levels in gastric mucose from rats. Data are mean ± SE values. For statistics, analysis of variance followed by Tukey's test was used. [a,b]Different letters represent significant differences between groups.

glucopyranoside, and quercetin-3-*O*-arabinopyranoside. The AqF, which did not exhibit gastroprotective action, contained only quercetin-3-*O*-galactopyranoside and quercetin-7-*O*-glucopyranoside. So these quantitative (almost 15 times more flavonoid content than found in EAF) and qualitative differences between fractions were essential to the cytoprotective effect observed with ME.

Although PGs play an important role in modulating gastric mucosal integrity and in regulating gastric acid secretion, little is known regarding regulation of PG synthesis by the stomach.[20] Arakawa *et al*.[36] suggested that PGs accelerate ulcer healing, possibly via angiogenesis, epithelial cell proliferation, reconstruction of extracellular matrices, suppression of inflammatory cell infiltration, and production of growth factors such as hepatocyte growth factor and transforming growth factor-β. In the present work, we observed that treatment with EAF induced a significant jump in PGE_2 production to double the basal levels. These results explain the gastroprotective action obtained from ME against ethanol as well as the antisecretory action observed under the pylorus ligature method. It is notable that our previous work with *A. castaneaefolia* extract also produced an increase in PGE_2 production.[8] However, more interesting results were obtained in the group of animals treated with EAF and pretreated with indomethacin (an inhibitor of cyclooxygenase enzyme). Results displayed in Figure 3 show that EAF was able to maintain a high PGE_2 level despite administration of indomethacin. These results may alter the therapy of gastroprotective drugs associated with NSAIDs because EAF was able to promote a sustained increase of PGE_2 levels, which is vital to the integrity of gastric mucosa. Furthermore, the association of EAF and NSAID was not capable of reducing levels of PGE_2 release in a significant manner. Park *et al*.[37] also evaluated flavonoids originating from *Scutellaria baicalensis* and observed that this compound provides a cytoprotective effect; they inferred dual action of wogonin on arachidonic acid metabolism, including the induction of cyclooxygenase-2 expression.

In conclusion, all these results taken together suggest that ME from the leaves of *A. triplinervia* did not show acute toxicity and exhibited an antisecretory property, anti-*H. pylori* effect, and gastroprotective action. The gastroprotective effect presented by the EAF was proven more efficient than that shown by the ME, while the gastroprotective action occurred by increasing the PGE_2 level.

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