Effects of Tea from *Turnera ulmifolia* L. on Mouse Gastric Mucosa Support the Turneraceae as a New Source of Antiulcerogenic Drugs

Juliano de Souza Gracioso,[a] Wagner Vilegas,[b] Clélia Akiko Hiruma-Lima,[c] and Alba Regina Monteiro Souza Brito*,[a]

Departamento de Fisiologia e Biofísica, Instituto de Biologia, Universidade Estadual de Campinas (UNICAMP),[a] Campinas, SP, Brazil, Instituto de Química de Araraquara, Universidade Estadual Paulista (UNESP),[b] Araraquara, SP, Brazil, and Departamento de Fisiologia, Instituto de Biociências, Universidade Estadual Paulista (UNESP),[c] Botucatu, São Paulo, Brazil. Received August 2, 2001; accepted December 10, 2001

Turnera ulmifolia **is a plant belonging to the family Turneraceae, popularly known in Brazil as chanana. This species is distributed from Guyana to southern Brazil where it is considered a weed. The plant occurs in tropical rain forest, fields, and gardens. Chanana tea is used in Brazilian folk medicine for the treatment of diseases related mainly to gastric dysfunction including gastric and duodenal ulcers. In this study, the ability of a lyophilized infusion, as an aqueous fraction (AqF) of the aerial parts of *T. ulmifolia*, was investigated for its ability to prevent ulceration of the gastric and duodenal mucosa was examined in mice and rats, respectively. The AqF significantly reduced the formation of lesions associated with HCl/ethanol administration by 39% and 46%, respectively, at doses of 500 mg/kg and 1000 mg/kg, *p.o.* The AqF also significantly reduced the incidence of gastric lesions induced by a combination of indomethacin and bethanechol by 58% and 72% at doses of 500 mg/kg and 1000 mg/kg, respectively. In stress-induced gastric ulcer, the inhibition by the AqF was 48%, 57%, and 58% at doses of 250 mg/kg, 500 mg/kg, and 1000 mg/kg, respectively ($p<0.05$). A pyloric ligature experiment showed that the highest dose of the AqF significantly affected the gastric juice parameters by increasing the pH from 2.5 (control) to 5.3 and decreasing the acid output from 11.3 (control) to 3.7 mEq/ml/4 h. The AqF had no significant effect on duodenal ulcers induced by cysteamine. Preliminary phytochemical screening confirmed that flavonoids were the major constituents of the AqF of *T. ulmifolia.* These results indicate that this extract has a significant antiulcerogenic effect, as popularly believed.**

Key words Turneraceae; ulcer; flavonoid: gastroprotective activity; *Turnera ulmifolia*

Flavonoids (or bioflavonoids) are a group of about 4000 naturally occurring compounds that are ubiquitous in vascular plants. These compounds are responsible for the autumnal burst of hues and the many shades of yellow, orange, and red in flowers. They are also important for the normal growth, development, and defense of plants.[1)] Flavonoids occur in several medicinal plants, and herbal remedies containing flavonoids have been indicated in folk medicine around the world.[2)] Thus these compounds are important for plants, animals, and humans.

The relationship between flavonoids and humans has stimulated the study of the biochemical, physiologic, and pharmacologic activities of these compounds. Flavonoids have antiinflammatory activity[3)] and protect the gastric mucosa against a variety of ulcerogenic agents in different mammalian species. As a result, many studies have examined the antiulcerogenic activities of plants containing flavonoids using either naturally derived or synthetic compounds.[2)] Despite some promising results obtained with this class of molecules, there are currently few flavonoid related to antiulcerogenic properties.

Turnera ulmifolia L., a plant popularly known in Brazil and South America as chanana, belongs to the family Turneraceae. This species is a small herb with a wide geographic distribution, ranging from Guyana to southeastern Brazil, including São Paulo state, where it is considered a weed.[4)] Some species of *Turnera* are used in folk medicine for the treatment of several diseases. Recently, Antonio and Souza Brito[5)] demonstrated in rats that organic extracts from *T. ulmifolia* inhibited gastric lesions induced by ligature of the pylorus, and by indomethacin and ethanol. Those authors also detected flavonoids in these preparations.

Since the tea of this species is used in folk medicine to treat gastric ulcers in Brazil and other South American countries, we investigated the antiulcerogenic properties of a lyophilized infusion obtained from the aerial parts of *T. ulmifolia*, in different models of gastric ulcer in mice. We also screened the extract for the presence of different classes of natural products to determine whether there was a correlation between the chemical composition of the infusion and the biological activity of the tea.

MATERIAL AND METHODS

Animals Fasted male Swiss albino mice (30—40 g) or male Wistar rats (200—220 g) from the Central Animal House of the State University of Campinas (CEMIB/UNICAMP) were used. The animals were fed a certified Nuvilab CR-a diet with free access to tap water, and were housed under standard conditions of illumination (12 h light/dark), humidity (60±1%), and temperature (21.5±2 °C). All of the experimental protocols were approved by the local Committee for Ethics in Animal Experimentation at UNICAMP.

Drugs All reagents were of the highest purity. The following drugs were used: absolute ethanol, acetic acid, bethanechol chloride, cimetidine, cysteamine, indomethacin, lansoprazole, sodium bicarbonate, (Sigma Chemical, St. Louis, MO, U.S.A.). The AqF was always dissolved in 0.9% NaCl and administered at doses of 100, 250, 500, and 1000 mg/kg *p.o.* or intraduodenally, depending on the experiment. All solutions, reagents, and aqueous fraction (AqF) were prepared immediately before use.

* To whom correspondence should be addressed. e-mail: abrito@unicamp.br

Plant Material Specimens of *T. ulmifolia* were collected in 1999 in the city of Porto Nacional, Tocantins, and were identified and authenticated by Dr. Solange de Fátima Lolis at the Biology and Public Health Institute of the University of Tocantins, Brazil. A voucher specimen (number 0071) was deposited in the HTINS herbarium located in the same university.

Infusion Preparation Dried, powdered aerial parts of *T. ulmifolia* (50 g) were infused with 500 ml of boiling water to give a 10% solution. The infusion was allowed to cool to room temperature before being filtered and lyophilized. This procedure yielded 2 g of lyophilized aqueous extract and was repeated several times to obtain sufficient material for the biological assays. Lyophilized samples were homogenized to produce a final AqF and the resulting sample was stored under refrigeration until the experiment.

Hippocratic Screening and Acute Toxicity The signs and symptoms associated with the oral administration of the AqF (500, 1000, 2500, and 5000 mg/kg) were monitored in 8 mice per dose. The mice were examined 0, 5, 1, 2, 4, 8, 24, and 48 h after administration of the infusion to assess possible clinical or toxicological symptoms. The mortality rate was monitored for a period of 2 weeks, as described by Souza Brito.[6]

Antiulcerogenic Activity. Ethanol/HCl-Induced Gastric Mucosal Lesions These experiments were performed using the method of Mizui and Doteuchi,[7] with some modifications. Mice were fasted for 24 h and then given the AqF at doses of 100, 250, 500, and 1000 mg/kg. Lansoprazole (30 mg/kg) or vehicle (NaCl 0.9%) was administered orally to the control mice. Fifty minutes after the administrations, all mice received an oral dose of 0.2 ml of 0.3 M HCl/60% ethanol solution and were killed by cervical dislocation 1 h later. The stomach was inflated by injecting 2 ml of normal saline and then opened along the greater curvature before fix in 5% formalin for 30 min. The ulcerative lesion index (ULI) was calculated as described by Szelenyi and Thiemer.[8]

Hypothermic Restraint Stress-Induced Lesions The method of Levine[9] was used, with some modifications. Mice were fasted for 36 h and then immobilized in a restraint cage at 4 °C for 4 h. The AqF from *T. ulmifolia* (100, 250, 500, 1000 mg/kg), cimetidine (100 mg/kg), or saline was administered 1 h before the stress procedure. The mice were killed and the stomach was removed and opened along the greater curvature to determine the ULI as described above.

Indomethacin-Induced Gastric Lesions in Cholinomimetic-Treated Mice These experiments were carried out as described by Rainsford.[10] In this model, gastric lesions were induced by administering indomethacin (30 mg/kg, s.c.) and bethanechol (5 mg/kg, i.p.); the drugs were administered to mice after a 36 h fast. The AqF (100, 250, 500, 1000 mg/kg), cimetidine (100 mg/kg, *p.o.*), or saline was administered 30 min before the induction of gastric lesions. The mice were killed 4 h after treatment with the ulcerogenic agents, and the stomachs were removed and inflated with 4% formalin in buffered saline to determine the ULI as described above.

Determination of Gastric Secretion This assay was performed using the method of Shay *et al.*,[11] with minor modifications. All groups of mice were fasted for 36 h, with free access to water. Immediately after pyloric ligation, the AqF (100, 250, 500, 1000 mg/kg), cimetidine (100 mg/kg), or saline was administered intraduodenally. The mice were killed 4 h later, and the abdomen was opened and the stomachs removed. The amount of gastric juice (mg) and the pH were determined. The total acidity of the gastric secretions was determined by titration with 0.01 N NaOH to pH 7.0.

Cysteamine-Induced Duodenal Ulcers Acute duodenal lesions were induced in four groups of rats according to the method of Szabo.[12] The rats received two oral administrations of cysteamine HCl (400 mg/kg) in 1 ml of distilled water at a 4 h interval to produce the duodenal lesions. The AqF (500, 1000 mg/kg), cimetidine (100 mg/kg), or saline was given orally 1 h prior to cysteamine challenge. All rats were killed 48 h after the first administration of cysteamine and the duodenum was removed and scored for the presence of lesions.

Chromatographic Analysis of the AqF from *T. ulmifolia* The chemical constituents present in the lyophilized infusion of *T. ulmifolia* were identified according to the method of Wagner *et al.*[13] The chromatographic analyses were done on glass plates (5—30 cm) coated with Fluka F254 silica gel 0.25 mm thick and eluted in three different solvent systems: *n*-butanol/acetic acid/water (BAW) 65 : 15 : 35 v/v; chloroform/methanol/ammonia 8 : 2 : 0.5 v/v; and chloroform/methanol/*n*-propanol/water 5 : 6 : 1 : 4 v/v (lower layer). The AqF (10 mg) was dissolved in 1 ml of a 1 : 1 v/v mixture of methanol–water, submitted to an ultrasonic bath for 5 min, and centrifuged. Approximately 10 μl of the solution was spotted onto the TLC plate. Alkaloids were detected by spraying the plates with Dragendorff's reagent or iodoplatinate. Anthraquinones were detected using 10% potassium hydroxide solution in methanol. Flavonoids were detected by their intense fluorescence in visible or UV light when developed with a natural product/polyethylene glycol (NP/PEG) reagent. General phenolic compounds were detected after exposing the plates to ammonia vapors and immediately observing the fluorescent spots under UV light. Saponins, triterpenes, and steroids were detected either with anisaldehyde–sulphuric acid reagent or sulphate–sulphuric acid solution, which produced a range of colors after heating for 5 min at 100 °C. Tannins were detected with 5% ferric chloride solution in methanol and with 1% gelatin solution. Standard solutions of rutin, isoquercetrin, chlorogenic acid, and catechin were prepared in methanol.

Statistical Analysis The results are expressed as the mean ± S.D. and significant differences in the ULI were determined by one-way analysis of variance (ANOVA) followed by Tukey's (among groups) or Dunnett's (relative to the negative control group) pairwise tests. ANOVA was used to analyze the linear fit of the dose-response curves, and the ED_{50} values were determined to confirm the dose dependence of the AqF effects. p values of less than 0.05 were considered significant in all analyses.

RESULTS AND DISCUSSION

Based on the phytochemical screening shown in Table 1, flavonoids were apparently present as secondary metabolites in the infusion of *T. ulmifolia* aerial parts. Other classes of products with no known antiulcer activity were also present as minor compounds.

Table 1. Phytochemical Screening of the AqF Obtained from the Aerial Parts of *T. ulmifolia*

Alkaloids	Anthraquinones	Flavonoids	Saponins, sriterpenes, steroids	Tannins	Phenolic compounds	Catechin	Rutin	Isoquercitrin	Chlorogenic acid
−	−	+	−	−	+	−	−	−	−

+, presence; −, absence.

Table 2. Effects of *T. ulmifolia* AqF and Lansoprazole on HCl/Ethanol-Induced Gastric Ulcer in Mice

Treatment	Dose (mg/kg)	ULI	Inhibition (%)	*r* value	*p* value	ED_{50} (mg/kg)
Control	—	34.7±9.5	—	—	—	—
Lansoprazole	100	9.0±5.1[a)]	76	—	—	—
AqF	100	32.7±8.8[b)]	5			
	250	25.4±4.9[b)]	18	0.6	8.2	196.2
	500	20.8±5.0[a)]	39			
	1000	18.4±6.4[a)]	46			

ULI analysis: ANOVA $-F_{(5,36)}=6.41$; Tukey test. $p<0.05$ for *a*) lansoprazole and AqF (500, 1000 mg/kg) *vs.* negative control, and *b*) AqF (100, 250 mg/kg) *vs.* lansoprazole ($n=7$).

Table 3. Effects of *T. ulmifolia* AqF and Cimetidine on Stress-Induced Gastric Ulcer in Mice

Treatment	Dose (mg/kg)	ULI	Inhibition (%)	*r* value	*p* value	ED_{50} (mg/kg)
Control	—	12.1±2.6	—	—	—	—
Cimetidine	100	3.9±1.6[a)]	68	—	—	—
AqF	100	13.4±2.7[b)]	10			
	250	6.3±2.1[a,c)]	48	0.6	4.9	759.8
	500	5.1±2.9[a,c)]	57			
	1000	5.0±2.0[a,c)]	58			

ULI analysis: ANOVA $-F_{(5,42)}=23.66$; Tukey test. $p<0.05$ for *a*) cimetidine and AqF (250, 500, 1000 mg/kg) *vs.* negative control; *b*) AqF (100 mg/kg) *vs.* cimetidine, and *c*) AqF (250, 500, 1000 mg/kg) *vs.* AqF (100 mg/kg), ($n=8$).

Numerous investigations have concluded that flavonoids play a central role in the antiulcer and antiinflammatory activities of plants.[2,3,14)] Recently, the antioxidant activity of flavonoids has attracted interest because of strong evidence that oxidation processes are involved in the mechanisms of several gastric disorders, including ulcerogenesis.[14,17)]

Preparations such as infusions, extracts, or fractions containing flavonoids as the major compounds generally produce no significant acute toxicity *in vivo.* In agreement with this, no signs or symptoms of acute toxicity were observed with any of the doses of the AqF. There were no significant differences in organ weight, in water or food intake or in the amount of feces produced by AqF-treated and control mice. None of the treated mice died during the 14 d of observation after the administration of the AqF. These findings indicate that the AqF from *T. ulmifolia* is safe in mice at doses of up to 5000 mg/kg *p.o.* However, the possible effects in other species have yet to be studied.

The antiulcerogenic activity of the AqF was assayed in distinct models of ulcerative gastric and duodenal lesions in mice and rats, respectively. Gastric acid is secreted from the parietal cells by a proton pump and is stimulated by acetylcholine, histamine, and gastrin.[18)] Anticholinergic and antihistaminergic drugs block the action of acetylcholine and histamine at muscarinic M_3 receptors and histaminergic H_2 receptors located in the basolateral membrane of parietal cells. In contrast, proton pump inhibitors such as lansoprazole act directly on H^+/K^+-ATPase, the final step in acid secretion, and therefore control acid secretion independently of the stimuli acting the parietal cells.[19,20)]

Prostaglandins are involved in the regulation of a variety of gastrointestinal functions, including blood flow and acid, mucus, and HCO_3 secretion. Mucus and HCO_3 released from the surface of epithelial cells are part of the mucosal defensive mechanisms and play an important role in protecting the gastroduodenal mucosa. Although the physiological regulation of mucus and HCO_3 release involves neuronal factors, prostaglandin E_2 is also important in the local control of this secretion.[18,20,22)] Some plant extracts containing flavonoids can inhibit H^+/K^+-ATPase proton pump activity or increase PGE_2 and mucus release, which could explain their antiulcerogenic action.[2)]

Mice pretreated with the AqF showed a significant reduction in ethanol induced gastric ulcers at doses of 500 and 1000 mg/kg (Table 2), although the effect was not dose dependent. These results indicate that compounds present in the AqF interfered with the ulcerogenic activity of ethanol.[23)] Cytoprotective drugs alone or in combination with antisecretory drugs have commonly been used to treat alcohol induced gastric ulcer in humans. Plants containing flavonoids are also effective in preventing this kind of lesion, mainly because of their antioxidant properties.[1,3)]

The AqF also significantly prevented the formation of stress-induced lesions at doses of 250, 500, and 1000 mg/kg (Table 3). Again, no dose dependence effect was observed, and there was no difference between the doses of 500 and

Table 4. Effects of *T. ulmifolia* AqF and Cimetidine on Indomethacin- and Bethanechol-Induced Gastric Ulcer in Mice

Treatment	Dose (mg/kg)	ULI	Inhibition (%)	*r* value	*p* value	ED_{50} (mg/kg)
Control	—	15.9±3.9	—	—	—	—
Cimetidine	100	8.4±4.0[a)]	76	—	—	—
AqF	100	14.3±3.2[b)]	10			
	250	11.1±3.2[b)]	29	0.790*	0.001	664.1
	500	6.6±1.7[a,c,d)]	58			
	1000	4.4±2.2[a,c,d,e)]	72			

ANOVA–Linerar fit $*p<0.05$; ULI analysis: $F_{(5,36)}=14.18$; Tukey test. $p<0.05$ for *a*) cimetidine and AqF (500, 1000 mg/kg) *vs.* negative control; *b*) AqF (100, 250 mg/kg) *vs.* cimetidine; *c*) AqF (500, 1000 mg/kg) *vs.* 100 mg/kg; *d*) AqF (500, 1000 mg/kg) *vs.* AqF (250 mg/kg), and *e*) AqF (1000 mg/kg) *vs.* AqF (500 mg/kg), ($n=7$).

Table 5. Effects of *T. ulmifolia* AqF Intraduodenally and of Cimetidine on the Biochemical Parameters of Gastric Juice in Pylorus-Ligated Mice

Treatment	Dose (mg/kg)	*n*	pH (units)	Gastric juice (mg)	Acid output 4 h (mEq/ml)
NaCl 0.9%	10 ml/kg	8	2.56±0.48	336±87	11.34±2.35
Cimetidine	100	8	5.56±1.03*	213±97	5.16±1.23*
AqF	250	8	3.37±0.78	295±76	8.92±1.61
	500	8	3.75±0.96	327±89	6.23±1.03*
	1000	8	5.37±1.81*	292±77	3.71±0.67*

Analysis of biochemical parameters: ANOVA $F_{(4,35)}$ pH=8.59; gastric juice=1.95; $[H^+]=14.89$, $p<0.05$. Dunnett test, $*p<0.001$ relative to the NaCl group.

1000 mg/kg, which produced 57 and 58% inhibition, respectively. The prevention of stress-induced lesions by the AqF most likely involved an action on gastric secretion.[20)] Antisecretory drugs such as cimetidine, lansoprazole, and other potent antisecretory drugs are used to treat this type of lesion in humans. However, various agents of vegetable origin with antioxidant properties or containing free radical scavengers have also demonstrated good activity when used to treat stress and ulcers induced by stress in rodents.[2,24)]

The gastric ulcers produced by the cyclooxygenase 1 and 2 inhibitor indomethacin[25)] given in combination with a parasympatomimetic drug were prevented by the AqF (Table 4) at doses of 500 and 1000 mg/kg. This time, the effect was dose dependent. Antisecretory drugs and cytoprotective drugs such as prostaglandin analogues or other agents that protect the gastric mucosa, alone or in combination, are generally used to treat this type of lesion in clinical practice. Various plants containing flavonoids are also able to increase gastric mucus and bicarbonate secretion as well as PGE_2 release from the gastric mucosa, thereby preventing the ulceration induced by NSAIDs in rats.[2,3,14)]

When the normal stomach motility was altered by ligation of the pylorus[26)] and cimetidine or the AqF was given intraduodenally, there was a significant increase in the gastric juice pH and decreased of acid output (1000 mg/kg) but no change in the amount of gastric juice was produced (Table 5). These data show again that a possible antisecretory effect is involved in the antiulcerogenic activity of the substances present in the AqF. The action of the AqF when administered intraduodenally agrees with the observation that flavonoids can be fully or partially absorbed from the gastrointestinal tract.[24)]

The AqF had no significant effect against duodenal ulcers, with only 17 and 25% inhibition of their formation occurring at doses of 500 and 1000 mg/kg, respectively (data not shown). However, a higher dose of purified flavonoids could have a significant effect in preventing such lesions in rats.

The antiulcerogenic activity of many plant species is related to their content of flavonoids. *Spartium junceum* L., a species used in Turkish folk medicine to treat gastric ulcer, contains glycosylated flavonoids with antioxidant activity.[27)] A flavonoid present in unripe plantain banana pulp (*Musa sapirntum* L.) protects the gastric mucosa from aspirin-induced lesions.[28)] Similarly, the flavonoid hypolaetin-8-glucoside present in several species of the genus *Sideritis* also has significant antiulcerogenic activity.[29)] The synthetic isoprenyl flavonoid Solon shows good antiulcerogenic and gastroprotective effects. This compound is derived from sophoradin, a flavonoid isolated from the traditional Chinese medicinal plant *Sophora subprostata.* The exact mechanism of its action remains unclear, but may involve an action on the production and metabolism of prostaglandins and mucus in the gastric mucosa.[30)]

Our data suggest that the effects of the *T. ulmifolia* infusion are probably related to an increase in prostaglandin or mucus formation, inhibition of H^+/K^+-ATPase of the gastric mucosa, or possibly to antioxidant and free radical-scavenging activity, since all of these mechanisms are related to flavonoid content in other species.[2,14,17,27)] Various compounds detected in minor concentrations in the AqF showed no antiulcer activity and did not belong to the classes of chemical substances described by Lewis[31)] as having antiulcerogenic properties.

Although the precise nature of the active components of the AqF is still not known, we have found that the flavonoids present in the AqF are C-glycosylated flavones derivated from luteonin and apigenin (Vilegas *et al*., unpublished data). The results of this study support the use of infusions of *T. ulmifolia* to treat general ulcers, and suggest that this species could provide useful phytotherapeutic agents for the treatment of gastric ulcers in humans.

Acknowledgments The authors thank Ana Claudia B. de Paula, Ana Beatriz A. de Almeida, Leônia M. Baptista, Luciana Magri, and Walber Toma for technical assistance. FAPESP and CNPq grants supported this work. J. S. Gra-

cioso is the recipient of a scholarship sponsored by FAPESP.

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