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Research Paper

Gastroprotective effects (in rodents) of a flavonoid rich fraction obtained from *Syngonanthus macrolepsis*

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Keywords
Eriocaulaceae; flavonoid; gastroprotective activity; medicinal plants; *Syngonanthus macrolepsis*

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Abstract

Objectives *Syngonanthus macrolepis*, popularly known in Brazil as '*sempre-vivas*', is a plant from the family Eriocaulaceae, it is found in the states of Minas Gerais and Bahia. The species contains a variety of constituents, including flavonoids with gastroprotective effect. In this work, a flavonoid-rich fraction (Sm-FRF) obtained from scapes of *S. macrolepis* was investigated for preventing gastric ulceration in mice and rats.

Methods The activity was evaluated in models of induced gastric ulcer (absolute ethanol, stress, non-steroidal anti-inflammatory drugs and pylorus ligation). The cytoprotective mechanisms of the Sm-FRF in relation to sulfhydryl (SH) groups, nitric oxide (NO) and antioxidant enzymes were also evaluated.

Key findings The Sm-FRF (100 mg/kg, p.o.) significantly reduced gastric injury in all models, and did not alter gastric juice parameters after pylorus ligation.

Conclusions The results indicate significant gastroprotective activity for the Sm-FRF, which probably involves the participation of both SH groups and the antioxidant system. Both are integral parts of the gastrointestinal mucosa's cytoprotective mechanisms against aggressive factors.

Introduction

Gastric ulcer is a common disease with multiple aetiologies, and it is highly prevalent worldwide.[1] It results from an imbalance between aggressive factors which promote gastric mucosal injury (acid, pepsin, *Helicobacter pylori*, non-steroidal anti-inflammatory drugs) and protective factors (bicarbonate, mucus, blood flow, prostaglandins), which are required for gastro-duodenal mucosal integrity.[2]

Several factors are involved in the pathogenesis of ulcers, including inadequate diet, genetic predisposition, altered acid secretion, rapid gastric emptying, defective mucosal defence mechanisms, psychological or physical stress and smoking.[3] Both *Helicobacter pylori* infection and continued use of non-steroidal anti-inflammatory drugs promote the generation of reactive oxygen species and have been reported to be important factors in the development of ulcers.[4]

Various classes of synthetic antiulcer drugs are used for pharmacological treatment of peptic ulcers (antacids, proton pump inhibitors, anticholinergic and histamine receptor antagonists).[5] However, the reported adverse effects and high cost of these drugs limits their use and stimulates the continuing search for an ideal antiulcer drug now extended to herbal drugs because of their availability, better protection, lower cost and lower toxicity.[6,7]

The Eriocaulaceae family comprises about 1200 species, which are distributed over 10 genera. It has pantropical distribution, but most species occur in near tropical regions, such as in the mountains of Venezuela or Brazil.[8–10] The family Eriocaulaceae is the dominant herbal family in the Cipó Mountain range in the state of Minas Gerais Brazil. The *Syngonanthus* genus is of economic importance, and

phytochemicals studies have revealed a rich presence of flavonoids.[11,12]

Research work has shown that medicinal plants promoved importants biological effects such as anti-inflammatory, antioxidant and gastroprotective effects.[13–15] Recent studies have shown gastroprotective activity promoted by varied extracts and fractions obtained from species belonging to *Syngonanthus* genus (*Syngonanthus bisulcatus* and *Syngonanthus arthrotrichus*) collected at Minas Gerais State, Brazil.[16–18]

The aim of the present study was to evaluate the gastroprotective activity of the (Sm-FRF) obtained from the scapes of *S. macrolepsis* in several induced-gastric ulcer models.

Materials and Methods

Animals

The experimental protocols were approved by the Committee for Ethics in Animal Experimentation. Male Swiss albino mice (30–40 g) or male Wistar albino rats (180–250 g) from the Central Animal House of the State University of Campinas were used. The animals were fed a certified Nuvilab CR-diet, in addition, had free access to water under fixed conditions of illumination (12/12 h light/dark cycle), humidity (60 ± 1.0%) and a temperature of (21.5° ± 1.0). Fasting was used prior to all assays because standard drugs were administered orally (by garage), or by intraduodenal route using a 0.9% saline solution (10 ml/kg) as the vehicle (negative control). The animals were kept in cages with raised wide mesh floors to prevent coprophagy.

Drug

The drugs and reagents were prepared immediately before use. The following drugs were used: cimetidine (Sigma Chemical Co, St Louis, MO, USA), lansoprazole (Aché, Brazil) and piroxicam (Sigma Chemical Co). The Sm-FRF was obtained from scapes of *S. macrolepsis* as described below and was administered at dose of 100 mg/Kg. All drugs and fractions were administered orally (gavage), or intraduodenally.

Plant material

Scapes of *S. macrolepsis* Silv were collected at Diamantina City, Minas Gerais State, Brazil, in 2002, and authenticated by Dr Paulo Takeo Sano of the Institute of Bioscience, University of São Paulo (USP), Brazil. A voucher specimen was deposited in the Herbarium of the Department of Botany, of the Institute of Biosciences, USP.

Preparation of the ethanolic extract and purified fractions

The powdered scapes (300 g) of *S. macrolepsis* were successively extracted by maceration with chloroform, ethanol and 80% ethanol (1 week for each solvent). The solvents were evaporated in vacuum to yield black syrups. A portion (2 g) of the ethanolic extract was submitted to chromatograph column on Sephadex LH-20 (100 cm × 5 cm), with methanol (MeOH) as the eluent. Fractions (10 ml) were collected and checked by thin power chromatography (TLC) on silica gel in Si gel plates using an, *n*-BuOH-AcOH-H_2O (65 : 35 : 25, v/v) solvent system natural products – Polyethlenglycol Reagent (Sigma Chemical Co) showing that fractions 1–22 were deficient in flavonoids, fractions 23–47 were intermediate fractions, and fractions 58–64 were rich in flavonoids.

Fractions 58–64 (60 mg) were further purified by HPLC System (Knauer) equipped with a refractive index detector (Knawer RI Detector D-14163) and Knawer register, L250E. The sample was injected in a Rheodyne injector (Knawer, version 0600) with a 100 µl sample loop. The RP 18 Phenomenex Luna columns (250 mm × 10 mm × 10 µm), was used. The mobile phase consisted of water (eluent A) and methanol (eluent B). The flux was 2 ml/min.

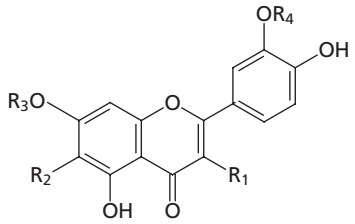
The isocratic mode was used with (80% B) to yield compounds: luteolin, 1 (13 mg, $t_R = 21$ min), and 6-hidroxyluteolin, 2 (10 mg, $t_R = 18$ min). Fraction 55 (12 mg) yielded pure compound luteolin-6-C-β-D-glucopyranosíde, 3, and fraction 68 (45 mg) yielded compounds 7-methoxyluteolin-6-C-β-D-glucopyranosíde, 4 (12 mg, $t_R = 9$ min), and 7,3′dimethoxyluteolin-6-C-β-D-glucopyranosíde, 5 (14 mg, $t_R = 12$ min) with 60% B as the eluent. (Figure 1).

The sample were analysed by TLC using SiF254 silica gel (Merck), and the solvent system $CHCl_3$: MeOH : H_2O (80 : 18 : 2, v/v), and *n*-BuOH : HAc : H_2O (65 : 35 : 25, v/v). The plates were examined under UV light (254 and 365 nm).

The Nuclear Magnetic Resonance (NMR) spectra of the pure sample were analysed in a Varian INOVA 500 spectrometer operated at 500 MHz for ^{1}H, and 150 MHz for ^{13}C. The DMSO-d_6 was used as solvent. The isolated compounds were identified by their NMR spectral data and compared to those reported in the literature.[19,20]

Gastroprotective activity

Several experimental induced-ulcer models were used to evaluate the gastroprotective effects of the Sm-FRF. An appropriate positive control group (lansoprazole, proton pump inhibitor or cimetidine, histamine receptor antagonists) was included in every assay.



Compounds	R1	R2	R3	R4	Names of the compounds
1	H	H	H	H	Luteolin
2	H	OH	H	H	6_hidroxyluteolin
3	H	glu	H	H	luteolin_6_C_β_D_glucopyranosíde
4	H	glu	CH_3	H	7_methoxyluteolin-6_C_β_D_glucopyranosíde
5	H	glc	CH_3	CH_3	7,3′ dimethoxyluteolin_6_C_β_D_glucopyranosíde

Figure 1 Compounds identified in the ethanolic extract of the *S. macrolepsis*.

Ethanol-induced gastric ulcer

Twenty-eight rats were randomly divided into four groups, and were fasted for 24 h prior to receiving an oral dose of 0.9% saline solution (10 ml/kg), lansoprazole (30 mg/kg), and Sb-FRF (100 mg/kg). After 60 min, all groups were orally treated with 4 ml/kg of absolute ethanol for gastric-ulcer induction. The animals were euthanized 1 h after the administration of ethanol, and the stomachs were then removed and opened along the greater curvature to quantify ulcerative lesions. The ulcerative lesion index (ULI) was calculated according to the methodology described by Szelenyi and Thiemer.[21] The experiment was performed as described by Morimoto *et al*.[22] with modifications.

Hypothermic restraint-stress ulcer

The gastroprotective activity of the Sm-FRF was assessed using the hypothermic restraint stress-induced gastric ulcer model with modifications.[23] After fasting for 24 h, the animals received a single oral administration of the Sm-FRF (100 mg/kg body weight), cimetidine (100 mg/kg) or 0.9% saline solution (10 ml/kg). At 30 min post-treatment, gastric ulceration was induced by immobilizing the animals in a closed cylindrical cage, maintained at 4°C. After 4 h, the animals were euthanized, the stomachs removed and then examined for ulcers as described previously.

Non-steroidal anti-inflammatory drugs-induced gastric ulcer

Gastric lesions were induced after 24 h of fasting with piroxicam (30 mg/kg, s.c.) administration, this, with either the Sm-FRF (100 mg/kg), cimetidine (100 mg/kg), or 0.9% saline solution (10 ml/kg) being orally administered 30 min before induction. At 4 h after treatment with the ulcerogenic agent, the mice were euthanized by cervical dislocation.[24] The stomachs were removed and examined for ulcers as described previously.

Pyloric ligation-induced gastric ulcer

The pyloric ligation-induced gastric ulceration assay was carried out according to the method developed by Shay *et al*.[25] In brief, Male Swiss albino mice (30–40 g) were fasted for 24 h, yet with free access to water until the start of experimentation. The animals were then randomly divided into four groups. At 30 min after intraduodenal administration of a single dose of either the Sm-FRF (100 mg/kg), cimetidine (100 mg/kg), or 0.9% saline solution (10 ml/kg) pylorus ligature was performed. Parameters such as gastric juice volume, pH and total gastric secretion acid content were determined. The stomach was removed, inspected internally, and its contents drained into a graduated centrifuge tube and centrifuged at 3000*g* for 10 min. The supernatant volume and pH were recorded with a digital pH meter. The total acid content of the gastric secretion was determined by titration to pH 7.0 with 0.01 N NaOH.

Ethanol-induced gastric lesions in N-ethylmaleimide pretreated rats

Rats were divided into group ($n = 5–7$) animals and fasted for 24 h. They were then treated intraperitoneally with N-ethylmaleimide (NEM) 10 mg/kg or 0.9% saline solution (10 ml/kg). Thirty minutes later, the different groups received an oral dose of the 0.9% saline solution or the Sm-FRF (100 mg/kg). After 60 min, all groups were orally treated with 4 ml/kg of absolute ethanol for gastric-ulcer

induction.[26] Animals were euthanized 1 h after the administration of ethanol by cervical dislocation and the stomachs excised and gastric damage determined as described previously.

Ethanol-induced gastric mucosal lesion in L-NAME-pretreated rats

Male Wistar rats were placed in fasting for 24 h and divided into three ($n = 5–7$) groups according to pretreatment: one group received the 0.9% saline solution (10 ml/kg), and two groups received Nω-L-arginine methyl ester (L-NAME) 70 mg/kg (i.p.), an oxide synthase blocking agent. Thirty minutes after administration, the groups were orally treated with 0.9% saline solution or the Sm-FRF (100 mg/kg). After 60 min, all groups were orally treated with 4 ml/kg of absolute ethanol to induce gastric ulcer.[27] The animals were euthanized 1h after the administration of ethanol by cervical dislocation, the stomachs excised and gastric damage determined as described previously.

Antioxidant activity

To assess the effect of the Sm-FRF (100 mg/kg) on the antioxidative system, we assayed the levels of glutathione peroxidase, glutathione reductase and superoxide dismutase in glandular stomach mucosa in rats with ethanol-induced gastric lesions. After treatment, the rats, were anesthetized with xylazine (50 mg/kg i.m.), ketamine (10 mg/kg i.m.) and euthanized by exsanguination via the abdominal aorta. The stomachs were removed, and the mucosae (from each stomach) were scraped off with glass slides, homogenized in phosphate buffer (0.1 M, pH 7.4) and frozen at −70°C until required for biochemical determinations.

Glutathione peroxidase activity

The glutathione peroxidase (GSH-Px) activity was assayed by following the decrease in absorbance at 365 nm induced by 0.25 mM H_2O_2 in the presence of reduced glutathione (10 mM), nicotinamide adenine dinucleotide phosphate (NADPH) (4 mM) and 1 U of glutathione reductase in phosphate buffer, pH 7.4. The changes in absorbance were read between 1 and 10 min, and the activity was expressed as pmol/min/mg of protein.[28]

Glutathione reductase activity

Glutathione reductase (GSSG-Rd), which reduces oxidized glutathione (GSSG), was assayed by the method of Worthington and Rosemeyer,[29] based on decrease in absorbance at 340 nm induced by oxidized glutathione in the presence of NADPH in phosphate buffer, pH 7.8. The decreases in absorbance were read between 1 and 10 min, and the activity was expressed as pmol/min/mg of protein.

Superoxide dismutase activity

Superoxide dismutase (SOD) was measured based on the reduction of nitroblue tetrazolium by a xanthine-xanthine oxidase system, which generates superoxide.[30] The decrease in absorbance at 340 nm was monitored for between 1 and 10 min, and the activity was expressed as U/mg of protein.

Statistical analysis

Results were expressed as mean ± SD or mean ± SEM Statistical significance between groups was determined by one-way analysis of variance (ANOVA) followed by Dunnett's tests, with $P < 0.05$ considered significant. The statistical software program utilized was GraphPad Prism® version 4 (USA, 2003).

Results

Ethanol-induced gastric ulcer

The effect of the Sm-FRF on ethanol-induced gastric ulcers in rats is shown in Table 1. Our results showed that the Sm-FRF (100 mg/kg p.o.), and lansoprazole (30 mg/kg) significantly reduced the ULI to 63 and 66% respectively when compared to the saline group (Table 1). These results suggest that Sm-FRF demonstrated gastroprotective activity against gastric lesions induced by absolute ethanol.

Hypothermic restraint-stress ulcer

Pretreatment with the Sm-FRF and cimetidine also significantly protected the gastric mucosa against stress-induced ulcers (6.7 ± 1.8** and 8.4 ± 3.2**, respectively) when compared with the saline group (27 ± 4) (Table 1). These results suggest that Sm-FRF protect the gastric mucosa from stress-related injuries.

Table 1 Effects of flavonoid-rich fraction (FRF) obtained from *S. macrolepsis* leaves on different models of acute gastric lesion induced in animals

Gastric lesion models	Treatment (p.o.)	Dose (mg/kg)	ULI (mm)	Inhibition (%)
Ethanol (rats)	Saline	–	102 ± 18	–
	Lansoprazole	30	45 ± 8.6**	66
	Sm-FRF	100	38 ± 2.1**	63
Stress (mice)	Saline	–	27 ± 4	–
	Cimetidine	100	6.7 ± 1.8**	79
	Sm-FRF	100	8.4 ± 3.2**	69
Piroxicam (mice)	Saline	–	19.6 ± 5.0	–
	Cimetidine	100	6.0 ± 3.0**	70
	Sm-FRF	100	8.6 ± 4.0**	56

ULI, ulcerative lesion index. ANOVA followed by Dunnett's test. Data are presented as mean ± S.D ($n = 5–7$). *$P < 0.05$, **$P < 0.01$.

Table 2 Effects of flavonoid-rich fraction (FRF) obtained from *S. macrolepsis* leaves on gastric juice parameters in rats submitted to pylorus ligature model

Treatments	Dose (mg/kg)	pH (unit)	Gastric juice (mg)	[H+] (mEq/ml/4h)
Saline	–	3.3 ± 0.49	248 ± 49	11 ± 0.81
Cimetidine	100	4.3 ± 0.48*	206 ± 44	6.9 ± 1.3**
Sm-FRF	100	3.3 ± 0.47	234 ± 26	12 ± 1.6

ANOVA followed by Dunnett's test. Data are presented as mean ± S.D (n = 5–7). *P < 0.05, **P < 0.01.

Non-steroidal anti-inflammatory drugs (NSAIDS)-induced gastric ulcer

According to the results obtained in this model, we observed that the Sm-FRF (100 mg/Kg), and cimetidine (100 mg/Kg) significantly reduced the ULI at 56 and 70%, respectively when compared with the saline group (Table 1), suggesting a gastroprotective effect against NSAIDs-induced gastric ulcer.

Pyloric ligation-induced gastric ulcer

The results obtained for gastric secretion restraint using the pylorus ligation model in rats showed that the Sm-FRF (100 mg/kg) given intraduodenally did not promote changes in the biochemical parameters of the stomach content, such as pH, concentration of H^+ ions and the volume of gastric juice after administration (Table 2). The results suggest that the gastroprotective effect exerted is not related to reduction of gastric acid secretion.

Ethanol-induced gastric lesions in NEM pretreated rats

From the model evaluating sulfhydryl group involvement in the gastroprotective effects of the Sm-FRF, we observed that when the groups were pretreated with NEM (blocker of sulfhydryl groups), and treated with the Sm-FRF, or saline, exacerbation of ILU occurred (80 ± 15** and 54 ± 15**, respectively) when compared to the controls (20 ± 8.4) (Table 3). The results demonstrate that the sulfhydryl compounds pathway is involved in the gastroprotective effect promoted by the Sm-FRF.

Ethanol-induced gastric mucosal lesion in L-NAME-pretreated rats

We observed that the groups pretreated with L-NAME and subsequently treated with the Sm-FRF (37 ± 17) did not display exacerbation of ULI when compared to the control group (34 ± 15), respectively (Table 3). The results suggest that nitric oxide (NO) is not related to the gastroprotective effects promoted by *S. macrolepsis*.

Table 3 Effects of the flavonoid-rich fraction (FRF) from *S. macrolepsis* on gastric lesions induced by ethanol in rats pretreated with NEM or l-NAME

Pretreatment (i.p)	Treatment (p.o.)	Dose (mg/kg)	ULI (mm)
Saline	Saline	10	20± 8.4
NEM (10 mg/Kg)	Saline	10	80 ± 15**
NEM (10 mg/Kg)	Sm-FRF	100	54 ± 15**
Saline	Saline	10	34 ± 15
L-NAME (70 mg/Kg)	Saline	10	59 ± 4.8*
L-NAME (70 mg/Kg)	Sm-FRF	100	37 ± 17

ULI, ulcerative lesion index. ANOVA followed by Dunnett's test. Data are presented as mean ± S.D (n = 5–7). *P < 0.05, **P < 0.01.

Table 4 Effects of flavonoid-rich fraction (FRF) obtained from *S. macrolepsis* leaves on the activities of antioxidative enzymes in the gastric mucosa of rats with ethanol-induced lesions

Treatments	Dose (mg/kg)	GSH-Px (pmol/mg protein/min)	GSSG-Rd (pmol/mg protein/min)	SOD U/mg protein
Normal	–	54.9 ± 5.3**	11.9 ± 1.3	7.9 ± 0.08**
Saline	10	23.0 ± 2.8	11.4 ± 1.5	13 ± 0.08
Sm-FRF	100	57.1 ± 5.3**	10.09 ± 2.3	7 ± 0.03**

GSH-Px, glutathione peroxidise; GSSG-Rd, glutathione reductase. ANOVA followed by Dunnett's test. Data are presented as mean ± S.E.M (n = 5–7). *P < 0.05, **P < 0.01.

Antioxidant activity

We observed a significant decrease in GSH-Px activity for the saline group, yet the Sm-FRF restored the levels of GSH-Px activity to normal in the gastric mucosa of treated rats. Hence, substances present in the Sm-FRF may well be associated with GSH-Px activity.

The activity of SOD increased in ethanol-treated rats (saline) compared with the normal group. Increased SOD activity was promoted by acute treatment with ethanol, yet was prevented by the Sm-FRF. There was no change in GSSG-Rd activity in any of the groups.

The results obtained in carrying out this experimental protocol demonstrated that the Sm-FRF did not alter GSSG-Rd and restored the activities of GSH-Px and SOD to normal levels (Table 4).

Discussion

Peptic ulcers are a common but serious disease worldwide and are one of the most important causes of morbidity affecting 10% of the world population.[31] Although many synthetic drugs are available to treat peptic ulcers, most of these drugs have adverse reactions when used long term.[4]

Medicinal plants have been reported in the literature as having a broad spectrum of biological activities.[32,33]

Anti-inflammatory and gastroprotective activities are attributed to the presence of chemical constituents present such as alkaloids, flavonoids and tannins.[34–36] For this reason, our research group has been interested in studying the gastroprotective effects promoted by natural compounds.[37,38]

Previous studies conducted by our research group with the species *S. arthrotrichus*[17] and *S. bisulcatus*[18] demonstrated significant gastroprotective effects promoted by the flavonoid-rich fraction at dose of 100 mg/kg. Therefore, the Sm-FRF (100 mg/Kg) was chosen for the study.

The gastroprotective and healing effects promoted by antioxidants drugs have been widely investigated in a number of studies. Alcohol intake has been shown to be associated with marked oxidative damage to gastric mucosa. Ethanol is widely used to induce experimental gastric ulcer in animals.[39]

Ethanol rapidly penetrates the gastric mucosa, and it causes membrane damage, cell exfoliation, erosion and ulcer formation.[40] Multiple action mechanisms including depletion of non-protein sulfhydryl concentration, modulation of the nitric oxide system and reduction of gastric mucosal blood flow are involved in the pathogenic process. Oxidative stress and depletion of antioxidants have also been considered crucial to alcohol-induced gastric ulcer.[41]

The results suggest that the Sm-FRF displays gastroprotective activity at the dose evaluated. This protection could reflect the inhibition of gastric secretion or an increase in protective substances release by the mucosa.

The next step of our study was to investigate the gastroprotective effect promoted by the Sm-FRF against gastric ulcerative lesions induced by acute stress and NSAIDs. These models involve the vagus nerve, as well as mucosal cytoprotective factors.

Disturbance of the microcirculation in the gastric mucosa, a change in gastric secretion, increased motility of the stomach, decrease in local prostaglandin biosynthesis, production of oxygen free radicals via the xanthine-xanthine oxidase system, neutrophils and lipid peroxidation initiated by reactive oxygen species have been suggested as mechanisms involved in stress-induced gastric ulcer.[42] Sm-FRF protect the gastric mucosa of mice from injuries related to stress. Is possible to suggest that Sm-FRF promote gastroprotective effect by cytoprotective and antioxidant activity to be investigated further.

According to the obtained results, we investigated a more specific model related to cytoprotection, which is the model of non-steroidal anti-inflammatory induced ulcer, using for this, the Sm-FRF (100 mg/kg) in mice.

NSAIDs, such as piroxicam, produce gastric damage in humans and experimental animals. The ulcerogenic effect of NSAIDs has been related to the potential of this drug to inhibit the synthesis of PGE_2.[43] Multiple actions such as augmented acid secretion, microcirculatory disturbances, increases in neutrophil infiltration, free radical formation and disruptions in the balance between NO expression and apoptosis contribute to this pathogenic process.[44] It was observed that Sm-FRF significantly inhibited ulcerative lesions induced by non-steroidal anti-inflammatory. This suggests gastroprotective effect promoted by cytoprotective mechanisms, because the lesions promoted by NSAIDs involve the inhibition of prostaglandin, the main mediators of cytoprotection in the gastric mucosa.

The pylorus ligature model brings changes in biochemical parameters of the gastric content such as pH, total acidity and gastric volume. It can be regarded as similar to the anti-secretory activity of substances affecting gastric acidity.[45] The accumulation of acid and pepsin in this model leads to auto digestion and ulceration of the gastric mucosa.[25,46] Sm-FRF did not change in the biochemical parameters of gastric secretion, but the anti-secretory cannot be totally ruled out because have not been evaluated other models such as the assessment of pump H + , K + ATPase, strong indicator anti-secretory activity.

Given the results, it became necessary to investigate the action of the mechanisms involved in the gastroprotective effect of the Sm-FRF. Therefore, we carried out experimental protocols to assess the contribution of sulfhydryl groups, nitric oxide and antioxidant enzymes.

Non-protein sulfhydryl compounds (NP-SH) play a key role in protecting the gastric mucosa against ethanol-produced injury.[47,48] Gastric damage is related to decreases in NP-SH concentrations since SH groups bind to free radicals formed by ulcerogenic agents that cause oxidative damage, cell death and epithelial erosion.[2] NP-SH levels also seem to be critical for the gastroprotective activity of some cytoprotective drugs.[49] The results suggest that the gastroprotection produced by Sm-FRF involves the participation of sulfhydryl compounds.

Nitric oxide participates in gastric defence mechanisms by regulating gastric mucosal blood flow, acid and alkaline secretion, mucus secretion and gastric mucosal blood flow.[50] To assess the involvement of nitric oxide in the gastroprotective mechanism of the Sm-FRF (100 mg/kg), we used a model for ethanol-induced ulcers in rats, pretreated with Nω-nitro-L-arginine methyl ester (L-NAME), a drug blocking NO synthesis. Thus, the gastroprotection promoted by Sm-FRF does not involve the participation of NO.

Reactive oxygen species (ROS) are important factors in ethanol-induced mucosal damage.[51] The role of antioxidants in the prevention and healing of gastric lesions has been widely investigated in a number of studies.[52,53]

Administration of absolute ethanol by gastric gavage induced marked damage to the gastric mucosa that was obvious during macroscopic examination. To explore the

effects of antioxidant defences on the ulceration process, antioxidants (GSH-Px, GSSG-Rd, and SOD) were evaluated (levels) in the gastric mucosa of rats having been pretreated with the Sm-FRF prior to ethanol administration.

The activity of SOD increased in ethanol-treated rats (saline) compared with the normal group. These results agree with Repetto *et al*.,[54] who indicated that oxidative stress could either increase O^{-}_{2} levels, or increase activity of the enzyme.

Conclusion

In conclusion, these results further suggest the potential of *S. macrolepsis*, which has demonstrated gastroprotective activity, for use as an anti-gastric ulcer drug. The observed gastroprotective effects probably involved increases in endogenous SH compounds, helping to maintain antioxidative enzyme levels in the gastrointestinal mucosa, which protect against aggressive factors.

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