



Research Article

GASTROPROTECTIVE EFFECTS OF THE AQUEOUS EXTRACT OF *SIMAROUBAAMARA* AUBLET (*SIMAROUBACEAE*) STEM BARK ON EXPERIMENTALLY INDUCED GASTRIC ULCERS IN RODENTS

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Abstract: *Simaroubaamara* Aublet stem bark decoction has been traditionally used in Brazil to treat malaria, inflammation, fever, abdominal pain, diarrhea, wound and as tonic. The aim of this study was investigate the gastroprotective effects of the aqueous extract of *S. amarastem* bark (SAAE) on different ulcer models induced in rodents. The SAAE was obtained by decoction and analyzed by high performance liquid chromatography (HPLC). Acute ulcer was induced by ethanol and indomethacin and the chronic model by acetic acid. HPLC analyze quantified condensed and hydrolysable tannins. Oral administration of SAAE (100, 250 and 500 mg/kg) inhibited ethanol and indomethacin induced ulcers. In the chronic model, SAAE (500 mg/kg) promoted re-epithelization, increased cell proliferation and restored glandular secretory activity of the gastric mucosa. The SAAE showed significant gastroprotective effects in the ulcerogenic models induced in rats. It restored glandular secretory activity and probably prevented the oxidative damage at the same time that increased regenerative and reparative capacities of the gastric mucosa.

Key words: *Simaroubaamara*, ulcer, tannins, gastroprotection

1. INTRODUCTION

Peptic ulcer is one of the world's major gastro-intestinal disorders, embracing both gastric and duodenal ulcers and affecting 10% of the world population¹. The pathophysiology of peptic disease is attributed to the imbalance between aggressive factors like acid, pepsin, *Helicobacter* infection and the local mucosa defenses like bicarbonate secretion, mucus and prostaglandins. *Helicobacter pylori* infection, use of non-steroidal anti-inflammatory drugs-NSAIDs, emotional stress, alcohol abuse and smoking are the principal etiological factors associated with peptic ulcer².

Although the acid secretions suppressors have been used as basic therapy for the healing of ulcer, there has been a growing interest in the mechanisms by which the ulcer heals as well as in the possibility that the speed and quality of healing may be pharmacologically modulated. Ulcer healing requires angiogenesis in the granulation tissue at the base of the ulcer together with epithelial cells proliferation in the ulcer margin and subsequent restoration of glandular architecture³.

In traditional medicine, many plants have been used to treat gastrointestinal disorders, including gastric ulcers⁴. *Simaroubaamara* Aublet, popularly known as "praíba", "marupá" and "pau - paraíba", is a large tree that reaches up to 40 m height and 0.5 to 0.9 m diameter⁵. The use of *S. amara* has a long history in folk medicine of many countries⁶. Ethnopharmacological data refer to use of a cup of *S. amarastem* bark decoction, 2 - 3 times per day, to treat

malaria, inflammation, fever, abdominal pain, diarrhea, wound and as tonic^{6,7}.

Simaroubaamara pharmacological assays showed antimalarial action of its fruits⁸, amebicide and bactericidal activities (against *Shigella flexneri* and *Salmonella typhosa*) of its stem bark and its root bark showed moisturizing action in the human epidermis⁷.

In phytochemical screening studies conducted by Polonsky et al.⁹ were isolated alkaloids, triterpenes and quassinoids from *S. amara* stem bark. Isolated six new triterpenoids from the *S. amara* stem bark and two other compounds previously known (3-oxatirucalla-7, 24-dien-23-ol and niloticina)¹⁰. Coimbra¹¹ has also isolated alkaloids and tannins from the bark of this specie.

Despite the pharmacological properties of *Simaroubaamara* stem bark, its antiulcerogenic effect has not been investigated. Therefore, the present study evaluated the possible gastroprotective potential of the aqueous extract of *Simaroubaamara* stem bark (SAAE) on experimentally induced gastric ulcers in rodents.

2. MATERIALS AND METHODS

2.1 Chemicals

Catechin, epicatechin, gallic acid, ellagic acid, chlorogenic acid, pantoprazole and indomethacin were purchased from Sigma - Aldrich Chemicals Co. (St. Louis, USA). Ethanol and acetic acid (Vetec, Duque de Caxias, Brazil), lactate dehydrogenase (LDH) (Boehringer Ingelheim,

São Paulo, Brazil) and proliferating cell nuclear antigen (PCNA) from Abcam (Cambridge, USA).

2.2 Material plant

The stem barks of *Simaroubaamara* Aublet were collected in São João, Pernambuco, Brazil (08 ° 52 '33 "S and 36 ° 22' 01" The Gr) and identified at the Agronomic Institute of Pernambuco. A voucher specimen was deposited at the Dárdano de Andrade Lima Herbarium under number 85268.

2.3 Extract preparation

The stem barks of *Simaroubaamara* were collected in December 2010. The samples were shade dried for 48 hours and then placed in a circulating air oven at a temperature of 45 ± 2 °C to stabilize the residual moisture. At the end, the barks were ground in a Knives mill. The aqueous extract of *Simaroubaamara* stem bark (SAAE) was obtained from the decoction of the powder (10:100 w/v) using distilled water as extractor solvent for a period of 10 minutes. The aqueous extract was concentrated in lyophilizer. The yield of *Simaroubaamara* dried extract was 12.15 g per liter of the SAAE.

2.4 Phytochemical screening

2.4.1 Thin layer chromatography

The dry extract of *Simaroubaamara* stem bark was analyzed for the presence of hydrolyzable tannins (gallic and ellagic acids), tannins condensed (catechins), flavonoids, saponins, coumarins, phenylpropanoids, cinnamic acid derivatives, alkaloids, triterpenes/steroids, monoterpenes, sesquiterpenes, quassinoids, iridoids and sugars. The phytochemical profile was assessed in silica gel chromatographic plates (Merck ® art. 105553, UV 250 - 366nm) using mobile phases, reagents and appropriate standards¹².

2.4.2 High Performance Liquid Chromatography (HPLC)

HPLC conditions: chromatographic analysis to quantify tannins and chlorogenic acid in the extractive solution were conducted in liquid chromatograph Shimadzu® (UFLC, Japan) controlled by software LC Solution 1.0 and consisting of LC-20 AT pump, degasser DGU - 20A5, Sil-20A autosampler and detector diode array (DAD) SPD - M20A. It was used Restek® C18 column (250 mm x 4 mm, 5µm) maintained at 30 °C. The standards and samples were eluted using a mobile phase gradient consisting of methanol (A) and 0.5% acetic acid (pH 3.0) (B). The conditions were: 0 - 50 min (10 - 90% A and 90 - 10% B), 50 - 55 min (90 -10% A and 10 - 90% B), 55-60 min (10% A and 90% B). Flow rate of 0.8 mL / min and injection volume of 20 µL.

Samples preparation: the aqueous solution of dried extract from *Simaroubaamarastem* bark in the concentration of 1.6 mg/mL was used as extractive solution. For standards, it was prepared aqueous solution of catechin (1.6 mg/mL), gallic acid (0.16 mg/mL), ellagic acid (0.16 mg/mL), epicatechin (0.1 mg/mL) and methanolic solution of chlorogenic acid (0.05 mg/mL). The standard concentrations were used to construct their analytical curves. All samples

and standards were filtered through membranes of 0.22 µm (Millipore®) and injected in triplicate. The chromatograms were obtained at 290 nm.

2.5 Animals

Male Wistar rats (*Rattusnorvegicus* var. *albinus*) (aged 2 months, weighing 250 – 280 g) were obtained from the Department of Physiology and Pharmacology at the Federal University of Pernambuco (UFPE), Pernambuco, Brazil. The animals were kept under standard environmental conditions (22 ± 2 °C; 12:12 h dark/light cycle). Water and industrialized dry food (Labina®, Purina, Brazil) were available *ad libitum*. The experimental protocol was approved by the Animal Experimentation Ethics Committee of UFPE (Process n°. 026449), in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals.

2.6. Antiulcerogenic activity

2.6.1. Ethanol-induced gastric ulcer

After 16 h of fasting, the animals were randomly divided into five groups (n = 6/group) and were orally pretreated with the aqueous extract of *Simaroubaamarastem* bark (SAAE) (100, 250 and 500 mg/kg), a vehicle (water) and pantoprazole (40 mg/kg), one hour before administration of the ulcerogenic agent. Gastric lesions were induced using ethanol (70%, 0.5 mL/100 g, p.o). The animals were euthanized using thiopental (140 mg/kg, i.p.) one hour after induction of gastric lesions. Their stomachs were removed, by opening them along the greater curvature, the contents removed and the stomach mucosa gently washed with saline (0.9%) and examined for quantification of lesions. The gastric lesion area was measured using planimetry (mm²) in relation to the total area of the gastric corpus¹³.

2.6.2. Indomethacin-induced gastric ulcer

After 16 h of fasting, the animals were randomly divided into five groups (n = 6/group) and were orally pretreated with the aqueous extract of *Simaroubaamarastem* bark (SAAE) (100, 250 and 500 mg/kg), a vehicle (water) and pantoprazole (40 mg/kg). Gastric lesions were induced by subcutaneous administration of indomethacin 30 mg/kg, 30 minutes after the treatment¹⁴. The animals were sacrificed 6 h after indomethacin injection, their stomachs removed and inspected under magnification to determine the gastric lesions produced. The results were expressed as lesions, ulcers and total index, which were obtained from scores determined by various alterations in the gastric mucosa, considering the color, edema and hemorrhage, loss of mucus, petechiae or damage to the mucosa folds, and the number and size of necro-hemorrhagic lesions¹⁵.

2.6.3 Acetic acid-induced gastric ulcer

Chronic ulcer induction was based on described by Takagi et al.¹⁶ with some modifications. The animals were divided into 3 groups (n = 6), given a restricted solid food diet for 24 h and, after this, anesthetized in order to perform surgery to expose the stomach. 0.05 mL of 30% acetic acid was injected into the subserosal layer of the external wall of the stomach. One day after administration of acid, daily treatment began and the animals were treated orally once

daily for 14 days with water (control), pantoprazole (40 mg/kg) or SAAE (500 mg/kg). During this period, the possible toxic effects of SAAE were evaluated using such parameters as mortality, changes in body mass and macroscopic analysis of vital organs. On day 15, all groups were sacrificed, the stomachs removed, photographed and the surface area of gastric lesion determined by computerized planimetry (Software ImageJ[®]) and the data expressed in mm².

At the end of the treatment, the blood was also collected by retro-orbital puncture for biochemical analyze of lactate dehydrogenase (LDH).

2.6.3.1 Histological analysis

The stomach lesions induced by acetic acid in rats undergoing different treatments were located, sectioned, and set in 10% buffered formalin. After setting, the samples was washed with water, immersed in 70% ethyl alcohol for 3-4 days and embedded in paraffin. Five- μ m thick paraffin sections were taken and stained with hematoxylin/eosin (HE) and Periodic Acid-Schiff (PAS). Histological analysis of the gastric sections was carried out using an automatic microscopy system MICRO DIP[®] (KacilInc)¹⁷.

2.6.3.2 Immunohistochemical analysis

The immunohistochemical for proliferating cell nuclear antigen (PCNA) was performed in samples of rats' stomach embedded in paraffin. Sections of 4 μ m were obtained and incubated for 30 min with monoclonal antibody against the anti-PCNA protein. Initially, the samples were deparaffinized in xylene and hydrated. Then antigenic retrieval was performed in microwave oven at 100 °C, the slides were cooled to room temperature and endogenous peroxidase was blocked by the incubation in peroxidase blocking solution for 7.5 min. After cooling, the slides were incubated separately with primary antibodies for PCNA (anti-PCNA antibody [PC10] - Proliferation Marker (ab29) - Mouse monoclonal antibody, Abcam Inc), 1:100, 30 min, and with secondary antibody (Nichirei Biosciences Inc.), 1:200 for 30 min and then washed with phosphate buffered saline (PBS). After washing, slides were

incubated with diaminobenzidine chromogen solution (DAB), washed in water, counter-stained with hematoxylin, dehydrated and mounted. Cells reactive for anti-PCNA were identified by the presence of a dark reddish-brown chromogen in the nucleus of epithelial cells. The reactivity was indicated using the following scores: mild, moderate and strong reactivity¹⁷.

2.7 Statistical analysis

Statistical analysis was performed using Graph Pad Prism 5.0 software. The difference between groups was assessed by analysis of variance (ANOVA), followed, when necessary, by Newman-Keuls test. The significance level for rejection of the null hypothesis was always $\geq 5\%$.

3. RESULTS

3.1 Phytochemical screening

3.1.1 Thin layer chromatography

Phytochemical analysis of dry extract from *Simarouba amara* stem bark demonstrated the presence of hydrolyzable tannins (gallic and ellagic acids) and tannins condensed (Leucoanthocyanidins and proanthocyanidins). Leucoanthocyanidins are precursors of proanthocyanidins, class in which is inserted condensed tannins (catechin and epicatechin). Phenylpropanoids and cinnamic acid derivatives (cafeic acid) were also identified as well as traces of saponins, steroids and quassinoids.

3.1.2 High Performance Liquid Chromatography (HPLC)

HPLC analysis of extractive solution revealed the presence of chromatographic peaks consistent with the standards. Standard retention times were 5.78, 17.80, 18.57, 19.15, 32.6 min for gallic acid, chlorogenic acid, catechin, epicatechin and ellagic acid, respectively. For extractive solution were 5.76, 17.90, 18.62, 19.26 and 32.7 min, respectively (figure 1). The concentrations of the mainly metabolites present in the extract were gallic acid (16.47 μ g/mL of extractive solution), chlorogenic acid (3.83 μ g/mL), catechin (349.96 μ g/mL), epicatechin (16.43 μ g/mL) and ellagic acid (1.29 μ g/mL). Linear regression analysis indicated linearity of the method.

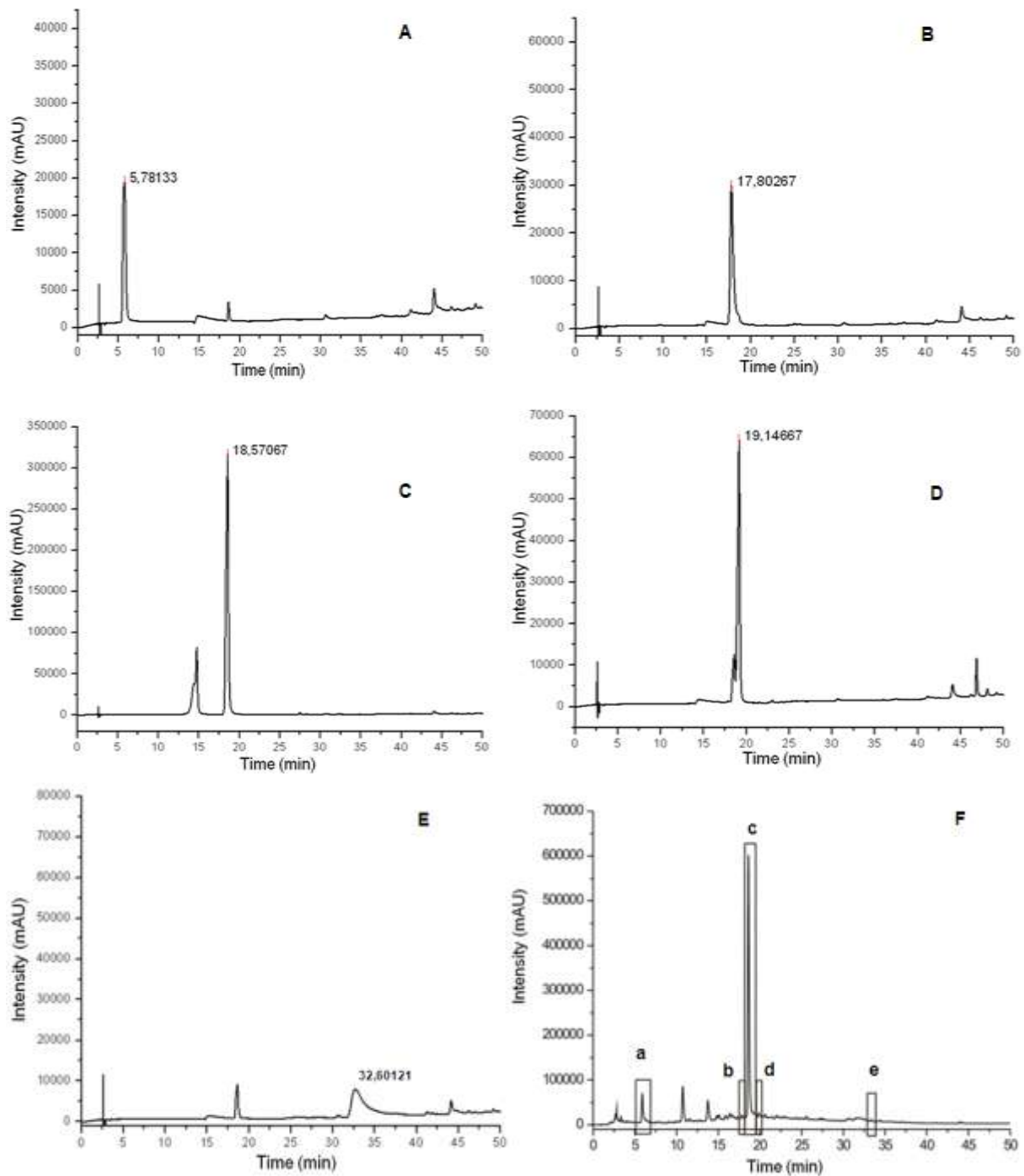


Figure 1: HPLC of standards and extractive solution of *Simarouba amarastem* bark. A – gallic acid; B – chlorogenic acid; C – catechin; D – epicatechin; E – ellagic acid, F – Extractive solution of *S. amara* (a – gallic acid; b – chlorogenic acid; c – catechin; d – epicatechin; e – ellagic acid).

3.2 Ethanol-induced gastric ulcer

The pretreatment with all doses of the SAAE inhibited the formation of gastric lesions (mm^2) induced by 70% ethanol compared to control (figure 2). The inhibition

percentage of ulcers was 66.9, 87.7 and 96.3% for doses of 100, 250 and 500 mg/kg, respectively. The inhibition showed by the highest dose was similar to pantoprazole (93%).

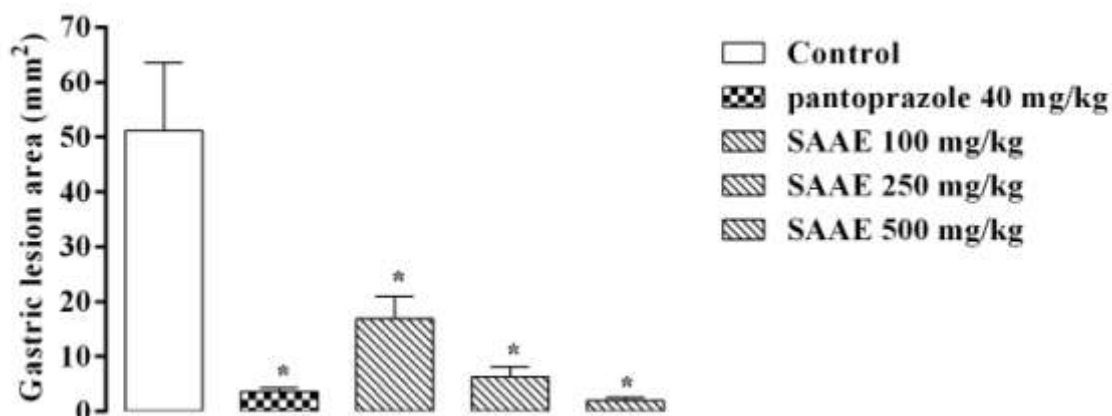


Figure 2: Gastroprotective effect of SAAE on gastric lesions (mm²) induced by 70% ethanol. The values represent mean ± S.E.M (n = 6 / group). * Statistically different from control (ANOVA followed by Newman-Keuls, p < 0.05).

3.3 Indomethacin-induced gastric ulcer

Indomethacin (30 mg/kg) produced a gastric lesions index of 3.99 ± 0.16; an ulcer index of 21.77 ± 0.93 and a total index of 25.76 ± 1.03 in the control group. Pretreatment of animals with SAAE at doses of 100, 250 and 500 mg/kg significantly reduced all indices as shown in table 1. The 100 mg/kg dose reduced the incidence of

lesions, ulcers and total indices by 62.0, 79.2 and 76.5%, the 250 mg/kg dose by 72.8, 85.4 and 83.4%, and the 500 mg/kg dose by 81.2, 94.8 and 92.7%, respectively. Pantoprazole reduced the lesions, ulcers and total indices by 77.0, 89.3 and 87.4% respectively, when compared with the control group.

Table 1: Gastroprotective effect of SAAE on gastric lesions induced by indomethacin (30 mg/kg, s.c.) in Wistar rats.

Parameters	Control	panto 40	SAAE 100	SAAE 250	SAAE 500
Lesion index	3.99 ± 0.16	0.92 ± 0.12*	1.52 ± 0.13*	1.08 ± 0.15*	0.75 ± 0.12*
Ulcer index	21.77 ± 0.93	2.33 ± 0.18*	4.52 ± 0.41*	3.18 ± 0.31*	1.12 ± 0.22*
Total index	25.76 ± 1.03	3.25 ± 0.71*	6.04 ± 1.50*	4.26 ± 1.05*	1.87 ± 0.18*

Panto 40: pantoprazole 40 mg/kg; SAAE (aqueous extract of *Simaroubaamarastem* bark) at doses 100, 250 and 500 mg/kg. The values represent mean ± S.E.M (n = 6 / group). * Statistically different from control (ANOVA followed by Newman-Keuls, p < 0.05).

3.4 Acetic acid-induced gastric ulcer

The SAAE (500 mg/kg) accelerated the healing of rats gastric ulcer during the 14 days of treatment (figure 3), whose main area of the lesion significantly decreased (1.89 ± 0.56mm²) (95.7%) compared to control group (44.51 ± 0.65mm²), however it did not differ of the reduction

promoted by pantoprazole (2.13 ± 0.83mm²) (95.2%). LDH levels (U/L) were also significantly decreased after the treatment of the animals with SAAE (573.70 ± 6.67) and pantoprazole (522.00 ± 8.97) compared to control (988.00 ± 8.97).

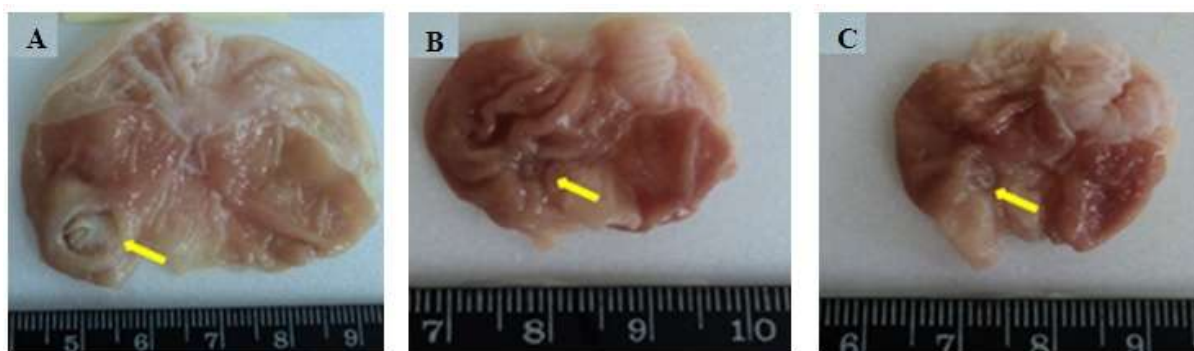


Figure 3: Gastroprotective effect of SAAE on gastric lesions (mm²) induced by 30% acetic acid. The values represent mean ± S.E.M (n = 6 / group). **A** – control; **B** – pantoprazole 40 mg/kg; **C** – SAAE 500 mg/kg.

There were no visible signs of toxicity (diarrhea or changes in behavior or locomotor activity) in animals treated with SAAE and pantoprazole. The animals treated with SAAE 500 mg/kg showed significant increases in body

mass gain (figure 4) and water intake (figure 5) compared to control only on the first week of treatment. Food intake remained high from day 5 until the end of treatment (figure 6) when compared to control.

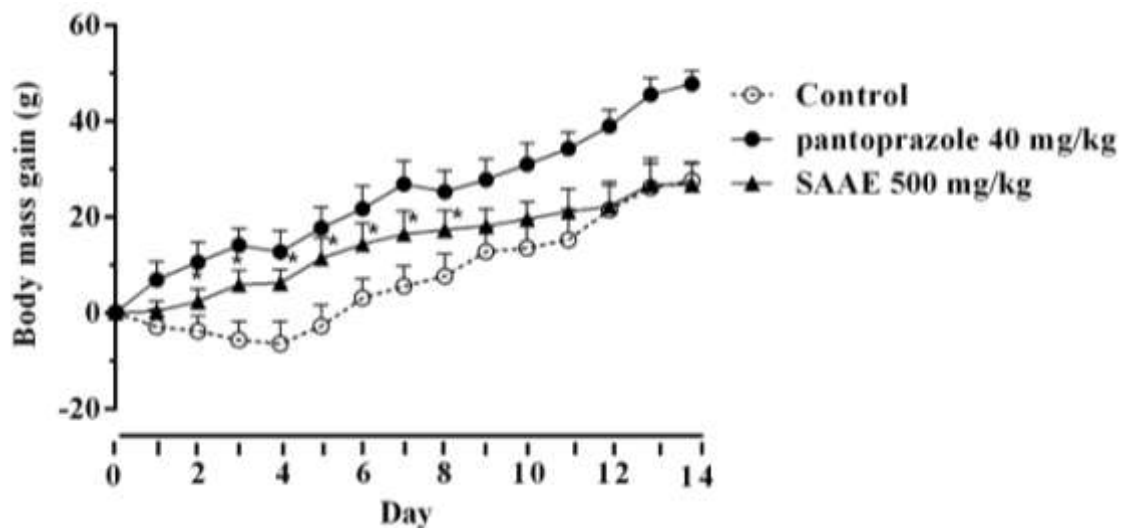


Figure 4: Body mass gain (g) of the animals treated with SAAE 500 mg/kg for 14 consecutive days. The values represent mean \pm S.E.M (n = 6 / group). * Statistically different from control (ANOVA followed by Newman-Keuls, p < 0.05).

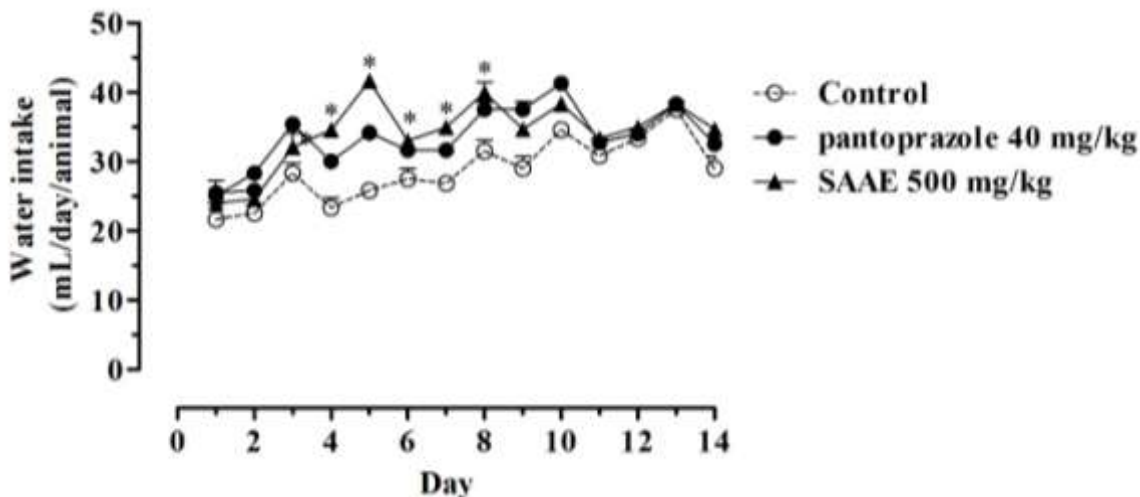


Figure 5: Water intake (mL/day/animal) of the animals treated with SAAE 500 mg/kg for 14 consecutive days. The values represent mean \pm S.E.M (n = 6 / group). * Statistically different from control (ANOVA followed by Newman-Keuls, p < 0.05).

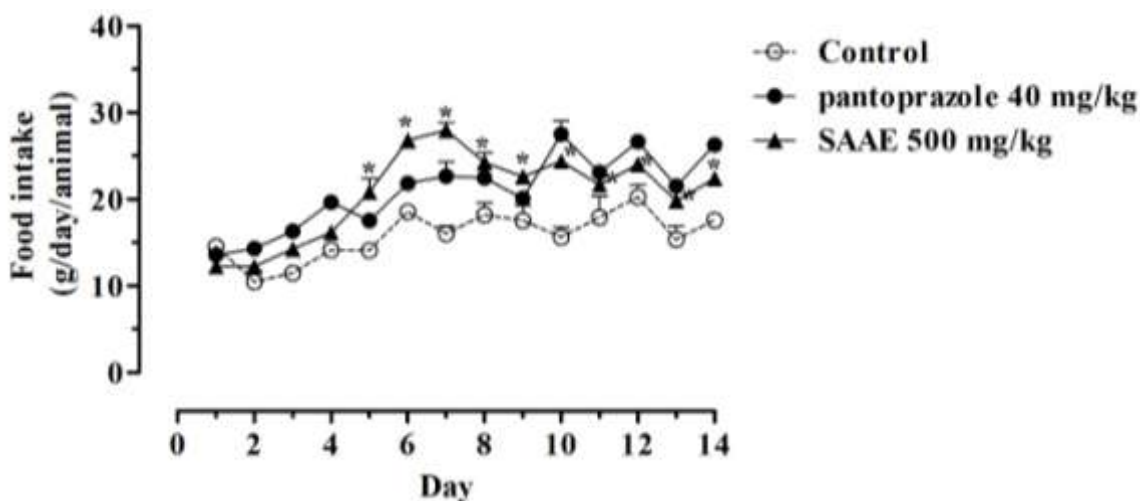


Figure 6: Food intake (g/day/animal) of the animals treated with SAAE 500 mg/kg for 14 consecutive days. The values represent mean \pm S.E.M (n = 6 / group). * Statistically different from control (ANOVA followed by Newman-Keuls, p < 0.05).

3.4.1 Histological analysis

The HE staining revealed that ulcer induced in the control animals by 30% acetic acid caused destruction of epithelial, mucosa and submucosa layers with abundant inflammatory infiltrate and confirmed the gastroprotective actions of pantoprazole (40 mg/kg) or

SAAE (500 mg/kg) after 14 days of treatment through visualized re-epithelialization areas. PAS staining also revealed the presence of gastric mucus and the arrangement of intact glands in the groups treated with pantoprazole or SAAE. The secretory activity is evidenced by the intense tone of pink (figure 7).

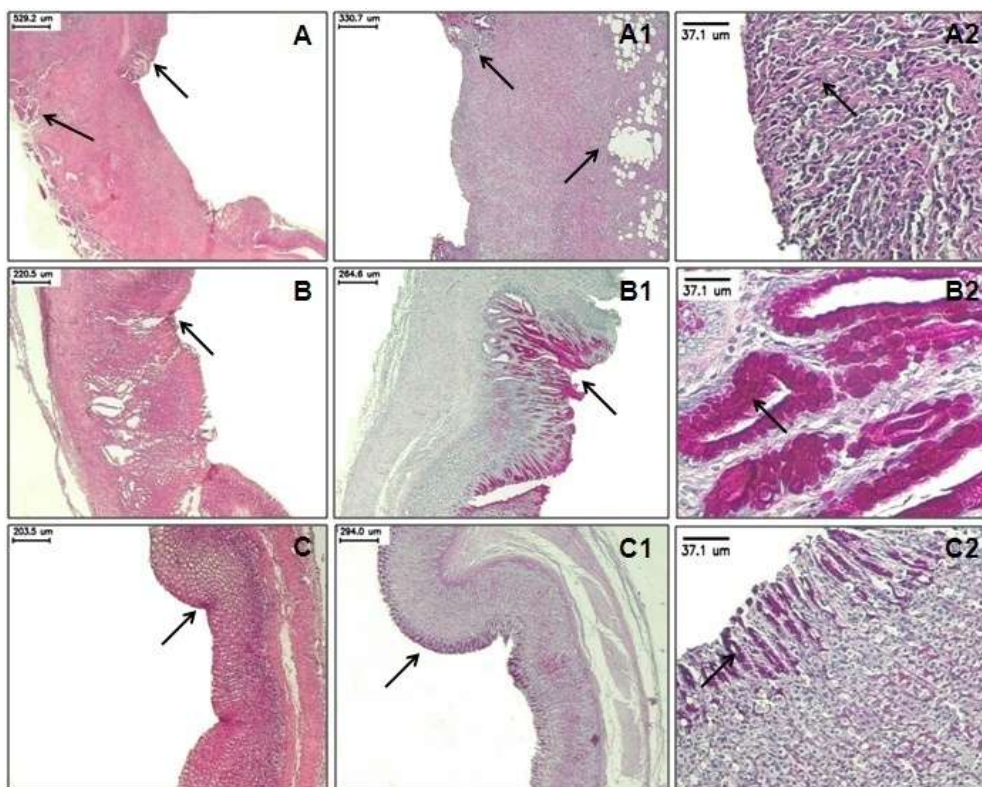


Figure 7: Histological analyze of rats' stomach treated with SAAE 500 mg/kg. **A** – HE stained control group (10x increased) showing destruction of epithelial, mucosa and submucosa layers (arrows). **A1 e A2** – PAS stained control with total destruction of the mucosa glandular layer (arrows) (10x increased) and abundant inflammatory infiltrate (arrows) (400x increased), respectively. **B** – HE stained pantoprazole group (10x increased) showing re-epithelialization areas (arrows). **B1 e B2** – PAS stained pantoprazole with re-epithelialization areas and glandular secretory activity (arrows) (10x and 400x increased, respectively). **C** – HE stained SAAE 500 mg/kg group (10x increased) with re-epithelialization areas (arrows). **C1 e C2** - PAS stained SAAE 500 mg/kg with re-epithelialization areas and glandular secretory activity (arrows) (10x and 400x increased, respectively).

3.4.2 Immunohistochemical analysis

The gastric tissues obtained in the acetic acid-induced gastric ulcer model were used for immunohistochemical localization of PCNA antibody. The PCNA-positive nuclei are marked by reaction with the color brown. The animals treated with the

SAAE showed intense immunoreactivity to PCNA (>75%) when compared to control. The reaction was similar to pantoprazole. The control samples showed intense lymphocytic infiltrate and a weak immunoreactivity to PCNA (<50%) that was observed only in the remaining epithelial cells nuclei in the layer adjacent to the ulcer (figure 8).

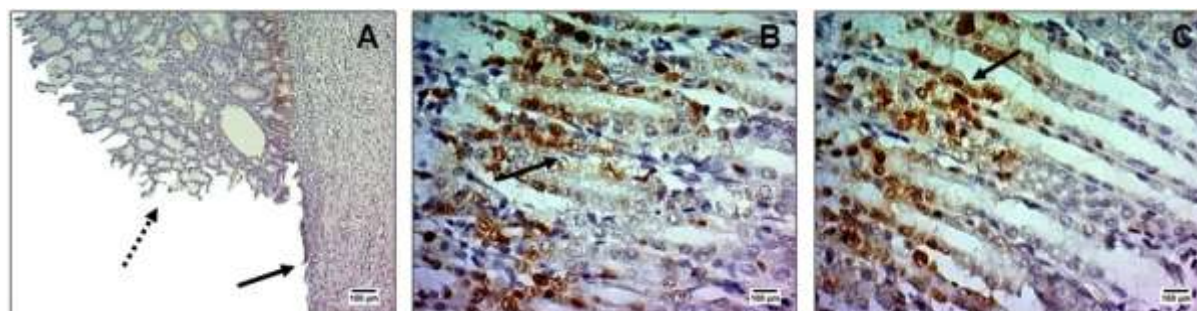


Figure 8: PCNA immunohistochemical analysis of rats' stomach treated with SAAE. **A** – control (200x increased): intense lymphocytic infiltrate and a weak immunoreactivity to PCNA (<50%) in the remaining epithelial cells nuclei (arrows); **B** – pantoprazole (500x increased): intense immunoreactivity to PCNA (> 75%); **C** – SAAE 500 mg/kg (500x increased): intense immunoreactivity to PCNA (> 75%).

4. DISCUSSION

Several plants extracts have been established as antiulcerogenics. However, this was the first study that investigated the gastroprotective properties of the aqueous extract of *Simarouba amara* stem bark (SAAE) and verified its antiulcerogenic action.

The HPLC analysis quantified the main metabolites observed in the preliminar phytochemical screening and the catechins were the major components quantified in the extractive solution. Complementary phytochemical data were provided in this study as the presences of hydrolysable and condensed tannins and chlorogenic acid derivate (cafeic acid). According to Kushima et al.¹⁸, phenolic compounds, which include the above cited, are apparently related to the interesting anti-inflammatory, wound healing, antioxidant and antiulcerogenic properties.

Plants rich in tannins have been traditionally used for their medicinal effects and several studies have demonstrated their anti-ulcer effects¹⁹. Studies showed that many tannins act as radical scavengers, intercepting active free radicals²⁰ and probably the SAAE would exert its gastroprotective effect due to tannins antioxidant actions²¹.

The treatment with SAAE was able to prevent gastric mucosa injury induced by 70% ethanol and indomethacin as well as accelerated the healing process in the chronic ulcer. Necrotic agents such as ethanol injured gastric mucosa through of mechanisms that involve blood stasis, formation of free radicals, lipid peroxidation, prostaglandins synthesis inhibition with consequent decrease in mucus production²².

Khennoufet et al.²³ analyzed the gastroprotective effects of acetone extract of *Quercus suber* and *Quercus coccifera* and their purified tannins on ethanol-induced gastric lesions in mice and rabbits. Both extracts and the purified tannins inhibited gastric secretion and the stomach lesions formation as well as significantly reduced lipid peroxidation in rabbits' brain homogenate. The authors suggested that the gastroprotective effects were also related to their anti-lipoperoxidant properties. Murakami et al.²⁴ showed that the ellagic acid was a potent competitive inhibitor of $K^+ - H^+ - ATPase$ pump and acted by competing with ATP hydrolysis local, thus significantly inhibited the HCl secretion.

The prostaglandins synthesis suppression induced by indomethacin increased the susceptibility of gastric mucosa lesions in the control group. All doses tested of SAAE reduced mucosal damage, suggesting a possible involvement of prostaglandins in the gastroprotective response. Mitjavila et al.²⁵ showed that low doses of tannic acid added to rat diet was also able to increase the amount of gastric mucus.

The chronic ulcer model induced by acetic acid is similar to human gastric ulcer not only by pathogenic characteristics but also by mechanisms and required time for healing²⁶. The SAAE (500 mg/kg) was able to promote re-epithelization and restore the glandular layer that had been destroyed by acetic acid administration. According to Vasconcelos et al.²⁷, the tannins precipitate microproteins at the peptic ulcer site forming a protective film which prevents the toxic substances absorption and the proteolytic enzymes action, promoting the healing natural process.

Besides this mechanism, our results showed that ulcer healing was also accelerated by increased proliferation of gastric cells in the SAAE treated animals viewed by PCNA intense immunoreactivity when compared to the control. PCNA is an auxiliary protein of *delta* and *epsilon* polymerases involved in DNA replication and repair. It is used as a cell proliferation marker²⁸. It is expressed in the G1 late phase and during S phase of the cellular cycle²⁹. A significant decrease in the LDH levels in the SAAE treated animals was also suggestive of tissue repair, since increased serum levels of this enzyme are observed in cases of cell injury and death³⁰.

Other information provided by this experimental model was related to some toxicological parameters recorded during two consecutive weeks of treatment, such as body mass gain and food and water intakes. The results showed that the SAAE did not compromise these parameters when compared to control.

5. CONCLUSION

The SAAE showed significant gastroprotective effects in the ulcerogenic models induced in rats. It restored glandular secretory activity and probably prevented the oxidative damage at the same time that increased regenerative and reparative capacities of the gastric mucosa.

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