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Prophylactic and Healing Activities of the Leaves Aqueous Extract of *Eremomastax speciosa* on Gastric Ulcers in Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author APA wrote the protocol, performed the statistical analysis and wrote the first draft of the manuscript. Authors CM and GST managed the literature and the biochemical analysis. Author ENZ managed the phytochemical analysis. Authors GEOE and PVT interpreted the histology slides and supervised the study. Author PVT designed the study. All authors read, corrected and approved the final manuscript.

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ABSTRACT

Aims: Information provided by practitioners of Cameroonian ethnomedicine suggested that *Eremomastax speciosa* (*E. speciosa*) possesses antiulcer activity. This led us to evaluate the prophylactic and healing effects of this plant.

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Place and Duration of Study: Department of Animal Biology & Physiology (Animal Physiology Laboratory) and Department of Organic Chemistry (Laboratory of Medicinal Chemistry), Faculty of Science, University of Yaoundé I, between May 2012 and November 2013.
Materials and Methods: The anti-ulcer activity of the leaf aqueous extract of *E. speciosa* was tested in rat using several acute gastric ulcer-inducing methods (HCl/ethanol, indomethacin-HCl/ethanol, indomethacin, absolute ethanol and cold/restraint stress). The healing effect on chronic acetic acid-induced gastric ulcers was also tested.
Results: Oral administration of the extract of *E. speciosa* prevented the formation of acute gastric lesions induced by different necrotizing agents. The inhibition was complete (100% inhibition) at the dose of 200 and 400 mg/kg of extract for the HCl/ethanol and cold/restraint stress methods, respectively. Pre-treatment with indomethacin significantly reduced the ability of the extract to inhibit the formation of HCl/ethanol induced lesions, with inhibition dropping to 39.63% for the dose 200 mg/kg. The lowest degree of gastric protection by the extract (12.13 to 13.80%) was obtained when indomethacin was administered alone by oral route. The protective effect of the extract (200 mg/kg) was reduced significantly when absolute ethanol (27.84% inhibition) was used as the necrotizing agent compared with the HCl/ethanol solution (100%). The prophylactic actions were associated with significant increases in gastric mucus production. The healing rate of chronic acetic acid-induced ulcers was 55.40 and 77.70%, for the dose 200 and 400 mg/kg of extract, respectively.
Conclusion: The prophylactic anti-ulcer effects of the extract are associated with enhanced mucus production which is an important factor in the mechanism of the healing process of chronic gastric ulcers.

Keywords: Eremomastax speciosa; gastric ulcers; cytoprotective activity; ulcer healing.

1. INTRODUCTION

Gastric ulcer is a break in the normal gastric mucosa integrity that extends to the muscularis mucosa layer or deeper [1,2]. It's a major disease of the gastrointestinal system which affects 5% of the world population with different etiologies [3]. It results from an imbalance between the aggressive (hydrochloric acid, pepsin, refluxed bile, leukotrienes, reactive oxygen species) and the protective (mucus-bicarbonate barrier, prostaglandins, mucosal blood flow, cell renewal and migration, and antioxidants) factors in the stomach [3,4]. The incidence varies with age, gender, geographical location and is associated with severe complications including haemorrhages, perforations, gastrointestinal obstruction and malignancy. Thus, this clinical condition represents a worldwide health problem because of its high morbidity, mortality and economic loss [1,2].

The prevention or cure of peptic ulcers is one of the most important challenges confronting medicine nowadays. Although there are many products used for its treatment, most of these drugs produce several adverse reactions [5]. However, plant extracts are some of the most attractive sources of new drugs and they have been shown to produce promising results in the treatment of gastric ulcers [6]. Clinical research has confirmed the efficiency of several plants for the treatment of gastrointestinal disorders, including gastric ulcers [7,8].

Eremomastax speciosa (Hochst.) Cufod. (Acanthaceae) is widely distributed in tropical Africa. It is a robust, polymorphous shrub that grows to 2 m high and it has a characteristic quadrangular stem and violate underside of the leaves, which has earned it the local name *Pang nyemshe*, meaning "red on one side" in the Bamileke region of Cameroon. This plant is used in Cameroonian ethnomedicine for the treatment of various stomach complaint and information from tradipractitioners suggests that it possesses antiulcer effects. Our previous studies showed that the methanolic extract of *E. speciosa* significantly reduced the formation of HCl/ethanol and cold/restraint stress ulcers, but was less effective against absolute ethanol. The extract also significantly reduced lesion formation and gastric acidity in the indomethacin/pylorus ligation model. It increased blood concentrations of antioxidant enzymes (catalase (CAT), Superoxide dismutase (SOD) and reduced glutathione (GSH)) and decreased malondialdehyde (MDA) concentrations in all models tested [9]. These results demonstrate the cytoprotective potential of the methanol extract but provide no indication of its ability to heal well-established chronic gastric ulcers. Moreover, WHO guidelines for the use of herbs for

medicines stipulate that preparation methods for scientific evaluation of medicinal plants should be as close as possible to the described ethnomedical procedure [10]. Thus, in this study, the aqueous extract of *E. speciosa* commonly used in ethnomedecine was used not only to evaluate its prophylactic potential against several experimental models of acute gastric ulcers, but also its ability to cure the chronic gastric ulcers.

2. MATERIALS AND METHODS

2.1 Material

2.1.1 Plant material

The leaves of *E. speciosa* were collected in May 2012 in Yaoundé (Centre Region of Cameroon) and identified botanically by Paul Mezili of the Cameroon National Herbarium (by comparison with existing voucher specimen number HNC/136984).

2.1.2 Experimental animals

Male albino Wistar rats (180 - 220 g) raised in the Animal house of the Animal Physiology laboratory, Faculty of Science, University of Yaounde I were used. They were fed with a standard laboratory diet (supplied by SPC Ltd, Bafoussam, Cameroon) and given tap water *ad libitum*. Prior authorization for the use of laboratory animals in this study was obtained from the Cameroon National Ethics Committee (Registration number FWA-IRB00001954). The use, handling and care of animals were done in adherence to the European Convention for the protection of vertebrate animals used for experimental and other purposes (ETS-123), with particular attention to Part III, articles 7, 8, and 9 [11].

2.2 Methods

2.2.1 Preparation of the plant extract

The leaves were chopped and quickly dried under the shade to avoid them getting moldy and then ground in a mechanical grinder to obtain 500 g a fine powder. This powder was extracted by infusion in 4 liters of boiled water for 15 minutes. After filtration through Whatman filter paper number 3, the filtrate was evaporated using a Raven ventilation oven (Jencons PLS, UK). The brownish solid obtained (77.7 g (15.5% yields)) was stored at 4℃. The extract was dissolved in distilled water and used for the subsequent experiments.

2.2.2 Ulcer preventive tests

2.2.2.1 HCl/ethanol-induced gastric lesions

The ulcer induction method described by Hara and Okabe [12] was used. The rats were deprived of food for 48 h prior to experimentation but had free access to tap water. The test rats received the extract of *E. speciosa* (100 and 200 mg/kg body weight (BW)) by oral route 1 hour before they were given the necrotizing solution of HCl/ethanol (150 mM HCl in 60% ethanol v/v). Positive and negative control rats received sucralfate (50 mg/kg BW) and distilled water (1 ml/200 g BW) respectively, in place of the extract. They were sacrificed 1 hour later using ether. The abdomen of each rat was opened and the stomach removed. The ulcers produced in the glandular region of each stomach were measured and scored as described by Tan et al. [13]. The ulcer index (UI), percentage of inhibition (% I) and percentage of ulcerated surface (% US) were calculated.

2.2.2.2 HCl/ethanol-induced gastric lesions in rats pre-treated with indomethacin

The effect of pre-treatment with indomethacine on the preventive effect of the extract on HCl/ethanol-induced gastric lesions was studied following the method described by Sun et al. [14]. All rats received indomethacin (20 mg/kg BW) by intra-peritoneal route. 1 hour later, the test rats received the plant extract (200 and 400 mg/kg BW) while the positive and negative control rats received sucralfate (50 mg/kg BW) and distilled water (1 ml/200 g BW) respectively by oral route. An hour later, all the animals were given orally 1 ml of HCl/ethanol solution. The rats were then sacrificed after 1 hour using ether and the stomach examined for gastric lesions.

2.2.2.3 Indomethacin-induced gastric lesions

Gastric mucosal lesions were induced by the method described by Pillai and Santhakumari [15]. After depriving the animal of food for 48 h, the test rats received the plant extract (200 and 400 mg/kg BW) by oral route, while the positive and negative control rats received sucralfate (50 mg/kg BW) and distilled water (1 ml/200 g BW), respectively. 1 h later, all the animals were given indomethacin (50 mg/kg BW) orally. They were sacrificed 5 h later and the ulcers produced in the

glandular region of the stomachs were measured and expressed according to the score described by Tan et al. [13].

2.2.2.4 Absolute ethanol-induced gastric lesions

Absolute ethanol-induced lesions were provoked using the HCl/ethanol-induced gastric lesions method. The rats received orally the plant extract, sucralfate or the vehicle 1 hour before absolute ethanol administration. They were also sacrificed using ether and the lesions formed were observed and scored.

2.2.2.5 Cold and restraint stress-induced gastric lesions

Stress-induced gastric ulcers were provoked in rats using the method of Tagaki and Okabe [16] described by Ohta et al. [17]. The animals were deprived of food (but not water) for 48 hours. Rats in the treatment groups were given the extract (200 and 400 mg/kg BW) by oral route while control rats received the vehicle or cimetidine. One hour later, the rats were placed in small individual wire cages and immersed in cold water (3-5℃), up to the level of the xiphoid. Two hours later the animals were sacrificed using ether and the stomachs were removed. The same protocol used with HCl/ethanol model for the assessment of lesion formation was performed.

2.2.3 Ulcer healing test

2.2.3.1 Acetic acid-induced chronic ulcers

Gastric mucosal lesions were induced by the method described by Pillai and Santhakumari [14] and has been described in details by Tan et al. [18]. Briefly, laparotomy was performed under light ether anaesthesia on experimental rats after a 24 h fast. Fifty micro litres of 30% glacial acetic acid were injected into the wall of the stomach corpus at the region of the lesser curvature, and the stomach wall wiped using cotton wool soaked in a 9‰ NaCl solution. The abdominal incisions were stitched up and feeding was resumed. Disinfectant (Betadine) was applied daily to avoid infection. Four days after the operation, a control group of five rats (4-day control) was sacrificed using ether, and their stomachs were opened in order to establish the degree of ulceration prior to the onset of treatment. The remaining rats were divided into four groups of five rats each and were treated by gavage during the next 10 days as follows: group 1 (vehicle control) received 1 ml/200 g of vehicle while groups 2 and 3 were given, respectively, 200 and 400 mg/kg of the extract. Group 4 rats were given 50 mg/kg of ranitidine (Azantac). An additional group of 5 healthy non-ulcerated rats was included but the rats were given neither the extract nor ranitidine. The rats were sacrificed and ulcer indices and gastric mucus production were measured. Whole stomachs were stored in 10% formaldehyde awaiting histological studies. Ulcer healing rates were calculated by comparing the ulcer status of extract- and ranitidine-treated rats with those of the ulcerated untreated controls (vehicle control). The degree of auto-healing was evaluated by comparing the untreated controls with the rats sacrificed on day 4 post-operation.

2.2.3.2 Mucus production assessment

Gastric mucus production was measured in the rats subjected to HCl/ethanol, indomethacin/HCl-ethanol, indomethacin, absolute ethanol, cold/restraint stress and acetic acid-induced ulcers. The gastric mucosa of each rat was gently scraped using a glass slide and the mucus obtained weighed using a sensitive digital electronic balance [19].

2.2.3.3 Preparation of histological sections

Sections of stomach walls perpendicular to the surface of each ulcer crater were made. Sections of normal stomach were also made for comparison. Haematoxylin and eosin stains of stomach sections were then performed following standard histological procedures described by Bayelet-Vincent [20] and the sections observed microscopically.

2.3 Statistical Analysis

Values in tables were given as arithmetic means ± standard error of the mean (SEM). The significance of differences between groups was analyzed by means of Analysis of Variance (ANOVA) followed by Student-Newman-Keuls comparison tests using Graph Pad Prism 5.03 software. All the differences were considered as significant for $P = 0.05$.

3. RESULTS

3.1 Ulcer Preventive Tests

Oral administration of the HCl/ethanol solution produced characteristic lesions in the glandular portion of rat stomachs. The aqueous extract of *E. speciosa* (100 and 200 mg/kg BW) significantly prevented the formation of gastric lesion (44.64 and 100% inhibition) induced by the HCl/ethanol solution. Sucralfate and extract at

the dose 200 mg/kg also completely prevented the formation of gastric lesions (100% inhibition). Treatment with the extract or sucralfate was associated with increased mucus production in comparaison with the control (Table 1).

The inhibitory effect of the extract and sucralfate against HCl/ethanol-induced gastric ulcers were suppressed when the rats were pre-treated with indomethacin. The prevention of lesion formation reduced from 100% to 39.63% at the dose 200 mg/kg of extract due to indomethacin pre-treatment (Table 2). This was accompanied by a reduction in mucus production from 319.43 mg to 89.35 mg at the same dose of the extract. Lesion inhibition was also reduced for sucralfate (41.92% inhibition).

Table 3 shows that when indomethacin was administered alone by oral route, the lowest degree of gastric protection (12.13 to 13.80%) was obtained both for the extract and sucralfate, with ulcer index (2.10 to 2.06) remaining close to those of control values (2.39). Treatment with extract or sucralfate was associated with a non-significant increase of mucus production compared with the control.

Table 4 shows the results obtained when the aqueous extract of *E. speciosa* was used to prevent the formation of gastric lesions induced using absolute ethanol. Gastric lesions observed were macromorphologically similar to those obtained using the HCl/ethanol solution. The protective effect of the extract reduced significantly when absolute ethanol was used as the necrotizing agent compared with the HCl/ethanol solution. The extract at the dose of 200 mg/kg provided only 27.84% inhibition compared to 100% with the HCl/ethanol method. Absolute ethanol considerably reduced mucus production values both in the controls and in the rats which received the extract compared with the HCl/ethanol solution.

Table 1. Effect of the leaf aqueous extract of *E. speciosa* on the HCl/ethanol-induced gastric lesions in rats

Treatment	Dose (mg/kg BW)	N	Ulcer index	Ulcerated surface (%)	Inhibition (%)	Mucus production (mg)
Control	-	5	3.92 ± 0.08	4.01	-	263,71 ± 13.74
E. speciosa	100	5	2.17 ± 0.21*****	1.51	44.64	286.79 ± 11.06
E. speciosa	200	5	0.00 ± 0.00******	0.00	100.00	319.43 ± 12.30*****
Sucralfate	50	5	0.00 ± 0.00******	0.00	100.00	309.99 ± 11.87

*Statistically different compared to control, *P = 0.05; **P = 0.01; BW = body weight; N = number of rats. The values are expressed as mean ± SEM*

Table 2. Effect of the leaf aqueous extract of *E. speciosa* on the HCl/ethanol-induced gastric lesions in rats pre-treated with indomethacin

Treatment	Dose (mg/kg BW)	N	Ulcer index	Ulcerated surface (%)	Inhibition (%)	Mucus production (mg)
Control	-	5	4.34 ± 0.50	4.59	–	76.03 ± 6.40
E. speciosa	200	5	2.62 ± 0.18**	3.38	39.63	89.35 ± 4.34
E. speciosa	400	5	1.71 ± 0.43***	1.50	60.60	105.81 ± 8.63**
Sucralfate	50	5	2.52 ± 0.21**	1.13	41.92	70.73 ± 3.30

*Statistically different compared to control; **P = 0.01; ***P = 0.001; BW = body weight; N = number of rats. The values are expressed as mean ± SEM*

Table 3. Effect of the leaf aqueous extract of *E. speciosa* on indomethacin-induced gastric lesions in rats

Treatment	Dose (mg/kg BW)	N	Ulcer index	Ulcerated surface (%)	Inhibition (%)	Mucus production (mg)
Control	-	5	2.39 ± 0.14	1.77	–	166.43 ± 9.37
E. speciosa	200	5	2.10 ± 0.05	1.53	12.13	174.90 ± 7.63
E. speciosa	400	5	2.06 ± 0.09	1.41	13.80	183.35 ± 5.90
Sucralfate	50	5	2.09 ± 0.21	1.23	12.55	168.97 ± 4.92

Statistically different relative to control; BW = body weight; N = number of rats. The values are expressed as mean ± SEM

Table 4. Effect of the leaf aqueous extract of *E. speciosa* on absolute ethanol-induced gastric lesions in rats

Treatment	Dose (mg/kg BW)	N	Ulcer index	Ulcerated surface (%)	Inhibition (%)	Mucus production (mg)
Control	-	5	4.85 ± 0.62	14.4	-	49.60 ± 5.60
E. speciosa	200	5	3.50 ± 0.39	5.30	27.84	70.60 ± 7.35
E. speciosa	400	5	2.93 ± 0.18*****	3.47	39.59	97.33 ± 8.88******
Sucralfate	50	5	3.48 ± 0.28	4.50	26.60	47.26 ± 7.54

*Statistically different compared to control, *P = 0.05; **P = 0.01; BW = body weight; N = number of rats. The values are expressed as mean ± SEM*

Table 5. Effect of the leaf aqueous extract of *E. speciosa* on cold/restraint-induced gastric lesions in rats

Treatment	Dose (mg/kg BW)	N	Ulcer index	Ulcerated surface (%)	Inhibition (%)	Mucus production (mg)
Control	-	5	3.37 ± 0.24	2.19	-	82.75 ± 5.21
E. speciosa	200	5	2.09 ± 0.17*******	1.07	37.98	114.53 ± 6.05
E. speciosa	400	5	0.00 ± 0.00*******	0.00	100.00	131.17 ± 11.54******
Cimétidine	50	5	0.00 ± 0.00*******	0.00	100.00	98.06 ± 10.47

*Statistically different compared to control, **P = 0.01; ***P = 0.001; BW = body weight; N = number of rats. The values are expressed as mean ± SEM*

The effects of subjecting the rats to a combination of restraint and cold stress are shown in Table 5. Control rats developed hemorrhagic lesions in the glandular portions of their stomachs 2 hours after cold immersion. *E. speciosa* extract (200 and 400 mg/kg BW) significantly prevented lesion formation, with complete inhibition of lesion formation at the dose of 400 mg/kg. Sucralfate (50 mg/kg BW) also completely prevented lesion formation. Mucus production increased significantly (P = 0.01), from 82.75 mg, in the controls to 131.17 mg (58.51%), at the highest dose of the extract.

3.2 Ulcer Healing Test

The ulcer healing test showed encouraging results after 10 days of treatment with *E. speciosa* extract. The macroscopic aspect of the stomachs showed deep and wide craters in the 4-day controls (Fig. 1B) representing an ulcerated area of 53.80 ± 9.09 mm^2. Spontaneous healing (in control rats treated with vehicle for 10 days) reduced the ulcerated area (Fig. 1C) to 29.60 ± 4.16 mm^2 representing an auto healing rate of 44.98%. However, this auto-healing was accompanied by a low degree of mucus production (52.83 mg) compared with the 4-day controls (66.92 mg). The extract of *E. speciosa* (200 and 400 mg/kg BW) and ranitidine (50 mg/kg BW) significantly reduced ulcer craters (Fig. 1D, 1E and 1F) after 10 days treatment to 13.20 ± 2.15 mm^2, 6.60 ± 1.03 mm^2 and 4.60 ± 1.08 mm^2, representing healing rates of 55.40%, 77.70% and 84.46%, respectively. The macroscopic reductions in ulcer crater size were accompanied by significant increases in mucus production in the extract-treated and sucralfate-treated groups (68.09 -91.13 mg) compared with the vehicle control (52.83 mg) (Table 1).

Histological presentation of the chronic acetic acid-induced ulcers is shown in Fig. 2. Histological section of normal rat stomach showed normal gastric mucosa, intact annular muscles, and longitudinal muscles in the muscularis (Fig. 2A). Histological gastric sections of 4^{th} day control rats showing the superficial loss of substance, glandular destruction, lymphocyte infiltration, and sclerosis. There was edema and fibrosis in the muscularis mucosa, and blood vessels presented intraparietal leucocyte infiltration, with necrosis and venous congestion. Histological gastric sections of vehicle control rats (Fig. 2B-D) showed signs of auto-healing, a beginning of regeneration of gastric glands and thickening of the serosa at the acetic acid injection site (Fig. 2E-F). In the rats treated with *E. speciosa* at 200 mg/kg showing amelioration of gastric tissues, with disappearance of fibrosis but with persistent edema and some glandular destruction (Fig. 2G-H). Stomach sections of extract-treated rats at 400 mg/kg showing normalization of the mucosa, without glandular destruction and with disappearance of fibrosis, sclerosis, and lymphocyte infiltration (Fig. 2I-J). Rats treated with ranitidine showed advanced scar tissue formation, healthy mucosa but with edematous muscularis (Fig. 2K-L).

Table 6. Healing effect of the leaf aqueous extract of *E. speciosa* on chronic acetic acid-induced gastric ulcers in rats

Treatment	Dose (mg/kg BW)	N	Ulcerated area (mm^2)	Ulcerated area (%)	Healing (%)	Mucus production (mg)
Control 1	-	5	53.80 ± 9.09**	7.97	-	66.92 ± 5.22
Control 2	-	5	29.60 ± 4.16	4.39	44.98	52.83 ± 4.79
E. speciosa	200	5	13.20 ± 2.15*	1.96	55.40	78.45 ± 6.47*
E. speciosa	400	5	6.60 ± 1.03**	0.98	77.70	91.13 ± 8.40**
Ranitidine	50	5	4.60 ± 1.08**	0.68	84.46	68.09 ± 4.88

*Control 1 (ulcerated rats sacrificed 4 days after acetic acid ulcer induction); Control 2 (ulcerated rats given vehicle for 10 days following ulcer induction). Statistically different compared to control 2, *P = 0.05; **P = 0.01; BW = body weight; N = number of rats. The values are expressed as mean ± SEM*

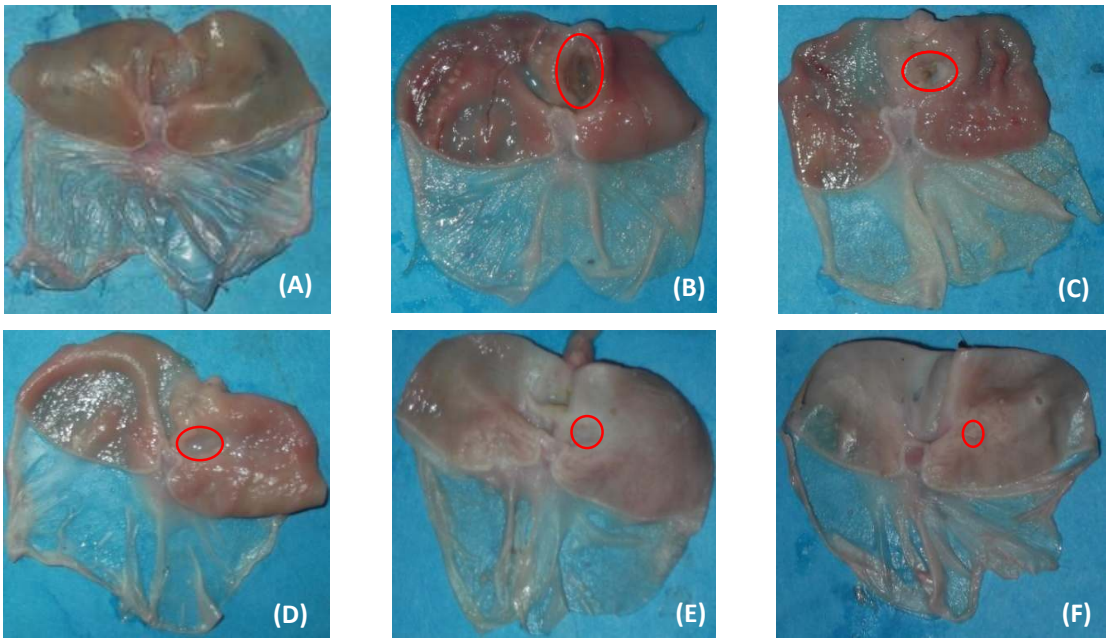

Fig. 1. Macroscopic aspect of chronic acetic acid-induced gastric ulcers

(A): Normal rat (Healthy control). (B): Control 1 (ulcerated rats sacrificed 4 days after acetic acid ulcer induction), (C): Control 2 (ulcerated rats given vehicle for 10 days following ulcer induction). (D) and (E): ulcerated rats treated with 200 and 400 mg/kg of extract, respectively, for 10 days after day 4 of ulcer establishment. (F): Positive control rat given ranitidine (50 mg/kg) for 10 days following ulcer establishment

4. DISCUSSION

Previous works revealed that the methanol extract of *E. speciosa* possesses cytoprotective and antioxidant activity in acute ulcers induced by various ulcerogens [9]. Other works demonstrated the anti-secretory mechanism of this plant [21]. The present study is investigating the prophylactic and healing activities of the aqueous extract of *E. speciosa* on several models of gastric damage. The results show that the aqueous extract of *E. speciosa* prevented, to varying degrees, the formation of gastric lesion induced by HCl/ethanol, indomethacin/HCl-ethanol, indomethacin and cold/restraint stress rats, and significantly enhanced the healing of chronic ulcers induced by acetic acid. These different necrotizing agents induce ulcers via different mechanisms. Thus, the effects of *E. speciosa* on these different mechanisms can allow us to explain the anti-ulcerogenic mechanism of this plant.

The mechanism of HCl/ethanol-induced gastric lesion involves the direct irritation of the stomach mucosa, the reduction of mucosal resistance and the erosion of the mucosal barrier. Pre-treatment with indomethacin exacerbated the irritant effect of the HCl/ethanol solution. The direct necrotic effect of indomethacin on the gastric mucosa is

more severe compared to the parenteral effects. Indomethacin is known to inhibit gastro-duodenal bicarbonate and endogenous prostaglandin (PGE) secretions [22]. The inhibition of PGE predisposes the stomach and duodenum to mucosal damage by irritants, whereas its stimulation can be protective [23,24]. Similar results obtained by Konturek et al. [25] were interpreted, suggesting that the anti-ulcer agent is acting through the intermediary of PGE which could be the case of our extract. In addition, this interpretation is supported by the significant increase of mucus production in extract-treated animals compared with the controls.

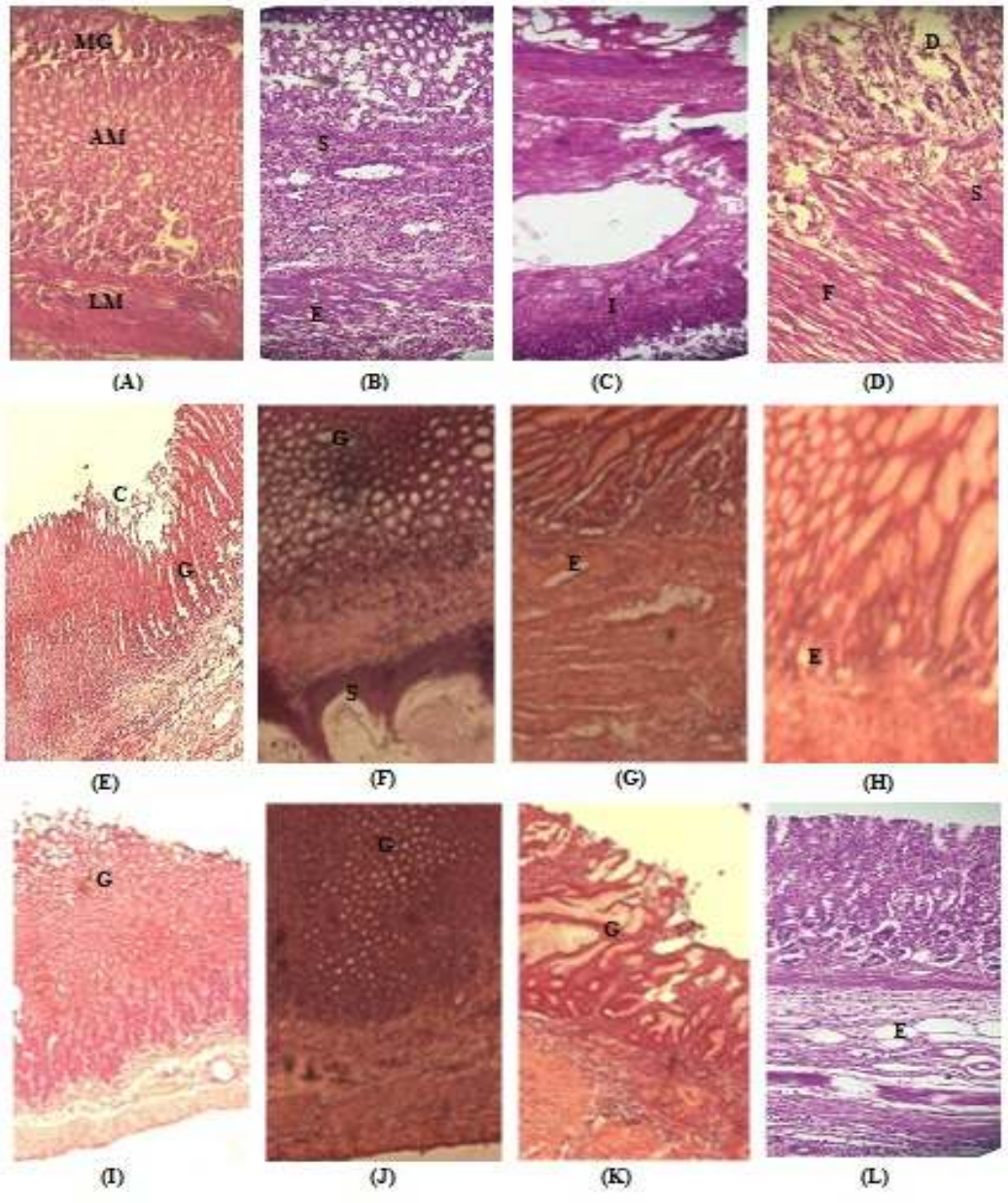

Fig. 2. Histological presentation of the chronic acetic acid-induced ulcers

(A): histological section of normal rat stomach; (B)-(D): Histological gastric sections of control 1 (4th day control); (E)-(F): Histological gastric sections of control 2 (vehicle control); (G)-(H): Stomach sections of rats treated with extract at 200 mg/kg; (I)-(J): Stomach sections of rats treated with extract 400 mg/kg; (K)-(L): Rats treated with ranitidine. GM: Gastric Mucosa, AM: Annular Muscles, LM: Longitudinal Muscles, D: Destruction, I: lymphocyte Infiltration, S: Sclerosis, E: edema, F: Fibrosis.

Absolute ethanol is highly corrosive to the gastric mucosa. Its pathogenic mode of action on gastric mucosa involves, in addition to superficial aggressive cellular necrosis, the release of tissue-derived mediators such as histamine and leucotrine C4. These mediators act on the gastric micro vasculature, triggering a series of events that result in mucosal and possibly sub mucosal tissue destruction [26]. The results of this study suggest that the aqueous extract of *E. speciosa* does not prevent the generation or the necrotic action of these mediators on the gastric micro vasculature. These results are similar to those obtained with the methanol extract of *E*. *speciosa* [9].

The mechanism of stress ulcer model involves an increase in intra-luminal gastric acid which causes mucosal damage by reducing the gastric adherent mucus [27,28]. The substances that reduce gastric acid secretion and improve gastric mucus secretion are useful in the prevention of stress ulcers. Thus the protective roles of mucus as a possible mode of action was evident as the results showed that *E. speciosa* extract significantly increased mucus production compared with the controls. In previous works [21], we have observed that the extract had antisecretory effects; thus the reduction of acid secretion could also be involved in the cytoprotective mechanism of this extract.

The pathogenesis of the acetic acid-induced chronic ulcers resulted from mucosal tissue necrosis. Tissue necrosis and subsequent release of arachidonic acid metabolites from injured cells, including leukotrienes B, attract leukocytes and macrophages, which then phagocytise the necrotic tissue and release pro-inflammatory cytokines. These cytokines in turn activate local fibroblasts, endothelial cells and epithelial cells to attempt a tissue restoration [29, 30]. Two weeks after ulcer induction, untreated experimental acetic acid-induced chronic ulcers undergo a certain degree of auto-healing (44.98% in the present experiment) due to the mucosal damage which constructively stimulates the secretion of growth factors in the adjacent mucosa [31].

In this study, *E. speciosa* extract promoted ulcer healing by shrinking the ulcer area and increasing the mucus covering of the gastric mucosa. The ulcer healing process is multi-staged and involves haemostasis, followed by inflammation and phagocytosis. The inflammation is characterized by an early intense neutrophilic infiltrate with the associated vascular dilation. This is followed by the proliferative stage manifested by angiogenesis, collagen deposition, wound contraction and epithelialization. The remodeling stage which follows consists of the formation of new collagen and increase in the tensile strength of the newly formed tissues [32-36]. Scar formation results in some loss of tissue functions in the scar area. However, where there is wound recovery, skin tissues aggregate from the area surrounding the wound to repair the exposed area, an action which has been attributed to myofibroblast activity [37]. It is evident therefore that the extract of *E. speciosa* promoted wound healing by influencing one or more of various cellular and molecular processes involved in the wound healing process. Macro-morphologically, the stomachs of rats that received 200 mg/kg of extract had significantly receded ulcer craters and a high mucus covering. In the rats that received 400 mg/kg of extract, there was complete healing without any signs of scar tissue formation and histological examination revealed significant proliferation of intact gastric glands with columnar epithelial cells and encroaching epithelial tissues. These observations suggest that the highest dose of extract promoted wound healing up to the recovery stage possibly through increase in the concentration and movement of fibroblasts surrounding the wound region or facilitation of the proliferation of epithelial cells to the uncovered area [38].

Many phytochemicals have been attributed anti-ulcerogenic properties [39] and the ulcer healing actions of various medicinal plant extracts have been demonstrated. Like as the case of *Musa sapientum* (plantain banana) [40] which promotes the healing of early ulcer dyspepsias and delays ulcer recurrences. The presence in plants of polar agents such as flavonoids and glycosides [41] has been shown to influence the healing process. Flavonoids and tannins have been shown to possess free radical scavenging and anti-inflammatory activity and promote regeneration and organisation of new tissue during the wound healing process [42,43]. Tannins, flavonoids, saponins, alkaloids and triterpenes have the ability to scavenge free radicals [44] and have been associated with gastric mucosal protection through increase of endogenous defence mechanisms [45-49]. The ability of *Sida corymbosa* to reduce the severity of haemorrhagic gastric lesions, to promote wound healing and to reduce inflammation was attributed at least one of the active constituents

or as a result of synergistic effects of these phytochemicals [50].

Previous works [21] revealed the presence of several classes of compounds of alkaloids, flavonoids, triterpenoids, phenols and tannins in aqueous extract of *E. speciosa*. The antioxidant effects of *E. speciosa* phytochemicals may be implicated in the promotion of wound healing. Oxygen free radicals play an important role in peptic ulcer injury and overproduction of reactive oxygen species results in oxidative stress, with detrimental cytotoxic effects that delay wound healing [51]. Levels of enzymatic (SOD, CAT, glutathione peroxidase (GPx), glutathione S-transferase (GST)) and non-enzymatic (ascorbic acid, vitamin E, glutathione) antioxidants have been observed to decrease in auto-healing wounds, indicating that wound creation results in loss of different free radical scavengers [52]. Neutrophil infiltration and lipid peroxidation in ulcerated gastric tissues have an inhibitory effect on the healing of acetic acid-induced chronic gastric ulcers in rats [53]. Therefore, elimination of reactive oxygen species by antioxidants have been shown to increase the amount of collagen and its organization indicating that antioxidants partly improve healing and could be an important strategy to improve healing of chronic wounds.

Amang et al. [9] in the previous works showed that, the methanol extract of *E. speciosa* significantly reduced the formation of experimental ulcers induced by cold/restraint, HCl/ethanol, indomethacin/pylorus ligation, and increased blood and gastric tissue concentrations of endogenous antioxidant enzymes (Catalase, SOD and GSH), with decrease in MDA concentrations in all models tested. Thus, the ability of the extract to improve the antioxidant status may also have been useful in the promotion of ulcer healing.

Peptic ulcer healing is accelerated by gastric acid inhibition, which enhances angiogenesis, cell proliferation, cell migration and maturation of the granulation tissue. In a previous experiment [21], the aqueous extract of *E. speciosa* has shown antisecretory effects which involve a mechanism common to both cholinergic and histaminergic pathways. In the present experiment, the cytoprotective action of *E. speciosa* aqueous extract against various ulcerogens is demonstrated. These results suggest that cytoprotection and gastric acid inhibition by *E. speciosa* extract can cause ulcer healing through enhancement of angiogenesis, cell proliferation, cell migration and maturation of the granulation tissue.

5. CONCLUSION

The results of this study show that the aqueous extract of *E. speciosa* prevents ulcers formation induced by various necrotizing agents (HCl/ethanol, indomethacin-HCl/ ethanol, indomethacin, absolute ethanol and cold/ restraint stress) and accelerates the spontaneous healing of acetic acid-induced chronic gastric ulcers. The anti-ulcer effects appeared to be associated with a reinforcement of gastric mucous defences through increased mucus production possibly mediated by PGE. The mechanism by which the extract accelerates the ulcer healing process includes, in addition of mucus production, antioxidant and antisecretory effects of the plant which were already reported. These results provide an endorsement for the traditional use of *E. speciosa* in the management of peptic ulcers. This statement is supported by the fact that the toxicity studies performed on the leaves aqueous extract of *E. speciosa* revealed it free and safe for consumption [54].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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