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Gastric ulcer healing effects of ethanolic extract of *Emilia praetermissa* Milne-redh (Asteraceae)

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Abstract
Emilia praetermissa is a plant which is used traditionally to treatment several diseases including gastric ulcers, and the ethanolic extract has reported cytoprotective anti-ulcer effects. The present study evaluated the healing effects of the ethanolic extract on chronic gastric ulcers using two chronic gastric ulcer models: glacial acetic acid and ethanol/aspirin solution-induced chronic gastric ulcer. Possible mechanisms of action were evaluated by testing the neutralizing effect (*in vitro*) of extract, and its cytoprotective activity against indomethacin in rats pretreated with L-NAME. The extract significantly healed chronic gastric ulcer induced by glacial acetic acid and by ethanol/aspirin solution (healing % = 85.92 and 100%, respectively, at the dose of 500 mg/kg). In neutralizing capacity tests, initial pH values at 25 °C were; sodium bicarbonate solution (9.28), gastric juice (2.90), and *E. praetermissa* extract (4.09). Incubation of sodium bicarbonate and gastric juice increased the pH of the gastric juice from 2.90 to 8.75 at 25 °C and from 2.06 to 8.20 at 37 °C. Incubation of *E. praetermissa* extract with gastric juice increased the pH of the gastric juice from 2.90 to 3.15 at 25 °C, and from 2.06 to 4.07 at 37 °C. Pre-treatment of rats with L-NAME significantly reduced the amount of nitrite in gastric mucus and further increased the ulcer index, but the extract reversed these effects. The extract of *E. praetermissa* possesses healing and anti-acid properties.

Keywords: *Emilia praetermissa*, ulcer healing, acid neutralizing capacity, L-NAME, nitrite

1. Introduction
Gastric ulcer disease is caused by the imbalance between damaging factors within the gastric lumen and protective mechanisms within the gastro duodenal mucosa. Although prolonged anxiety, emotional stress, hemorrhagic surgical shock, burns and trauma are known to cause severe gastric irritation, the mechanism is still very poorly understood [1]. However, it is established that major causative factors include rise in gastric acidity and peptic activity (linked to acetylcholine, gastrin and histamine), caffeine, alcohol, hydro alcoholic acid, sodium chloride, nonsteroidal anti-inflammatory drugs (NSAIDs) and stress. There are two major components to the ulcerogenic effects of NSAIDs in the stomach namely, their topical irritant effects on the epithelium and their ability to suppress prostaglandin synthesis. There is also a time- and dose-dependency of both suppression of gastric prostaglandin synthesis and ulcerogenic activity.
Treatment of gastric ulcers includes H_2- and M_1-blockers of histamine, proton pump inhibitors which decrease secretion of acid, and sucralfate and carbenoxolone which provide mucosal protection. Although these drugs have brought about remarkable changes in ulcer therapy, their efficacy is still debatable. Reports on clinical evaluation of these drugs show that there are incidences of adverse effects and drug interactions during ulcer therapy [2]. The usual medical treatment for peptic ulcer is either by the inhibition of acid secretion or by neutralization of the acid or by antibiotics (for example metronidazole, amoxicillin, clarithromycin, and tetracycline) and other drugs are extensively used in the management of peptic ulcers [3]. The neutralization of gastric acid can be done by antacid administration but their effectiveness is only for a brief period. Thus, there is a need for more effective and less toxic antiulcer agents. The multi-factorial genesis of gastro-duodenal ulcers has led to the discovery of novel and more efficient anti-ulcer therapies, and this has been accompanied by the development of a large number of experimental methods to evaluate their anti-ulcer activity as well as their mechanisms of action [4].

Plants as sources of new drugs have been shown to produce promising results for gastric ulcer treatment [5], and *E. praetermissa* (originally described from Sierra Leone and Nigeria and subsequently found in Cote d'Ivoire, Ghana, Guinea and Liberia) [6, 7] is traditionally used for gastric diseases. Qualitative phytochemical study revealed the presence of saponins, flavonoids, oils, phenols, coumarins, sterols, triterpenoids and polysaccharides. A previous study showed cytoprotective activity of the ethanolic extract of *E. praetermissa* in different gastric ulcer models, and possible risk of organ toxicity following chronic consumption at doses greater than 500 mg/kg [8].

In the present study, we studied the healing activity of the ethanolic extract of *E. praetermissa* on chronic gastric ulcers induced by glacial acetic acid and by ethanol/aspirin solution. The possible mode of action was studied by testing the gastric acid neutralizing capacity *in vitro* as well as cytoprotective action in rats pre-treated with L-NAME.

2. Materials and Methods

2.1 Plant Collection and Extraction

Plant collection was done in Yaoundé, Center Region of Cameroon, and identification by comparison with existing voucher specimen N°32105/SRF/HNC at Cameroon National Herbarium. Dried leaves were crushed, powdered and extracted with 95% ethanol (10% w/v) for 48 hours, filtered through Whatman filter paper No. 3, and the filtrate concentrated using a rotavapor, and then evaporated at 40°C using a *Raven* convection air oven (Jencons PLS, UK).

2.2 Experimental animals

Male albino Wistar rats (140-180 g) were used. The Cameroon National Ethics Committee (Reg. No. FWAIRB00001954) approved the experimental protocol. Animals, maintained under standard conditions in the animal house of the Animal Physiology Laboratory, were fed a standard laboratory diet and given fresh water *ad libitum*.

2.3 Acetic acid-induced chronic ulcers in rats

The method described by Pillai and Santhakumari (1984) [9] was used. Briefly, following a 24h fast, laparotomy was performed under ether anesthesia, and a solution of 30 % glacial acetic acid was used to create chronic gastric ulcers. Betadine was applied daily to the abdominal incisions to avoid infection. Four days post operation, a control group (control 1) was sacrificed and their stomachs opened to establish the degree of ulceration prior to the onset of treatment. Remaining rats were divided into four groups and treated with distilled water, extract (250 and 500 mg/kg), and sucralfate (50 mg/kg) for 10 days. After sacrifice, the stomachs were fixed and stored in formaldehyde awaiting histological studies.

2.4 Ethanol/Aspirin-Induced Chronic Ulcers

A modification of the method of Sun-Hye *et al.* (2008) [10] was used. Gastric ulcers were induced in rats following a 48h fast, using ethanol (70 %; 1 ml/200 g) and aspirin (200 mg/kg) by oral route. 24 hours after induction, ethanol (15 %; 1 ml/200 g) and the aspirin (200 mg/kg) were administered 4 times at 24h interval to maintain the ulcers. Ulcers were not induced in the normal control group *(untreated, non-ulcerated rats that received distilled water for 10 days)*. 24 hours after the last administration of ethanol/aspirin, rats of control group 1 were scarified and the remaining animals divided into four of groups of 5 rats each and treated once a day for 10 consecutive days as follows: 1 ml/200 g of vehicle; 250 and 500 mg/kg of extract, and 50 mg/kg of ranitidine.

Preparation of histological sections

Sections of stomach walls were made perpendicular to the surface of each ulcer crater. 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 (2002)[11] and the sections observed microscopically.

2.5 Evaluating the neutralizing effect *(in vitro)* of the extract

The neutralizing effect of the extract on gastric juice was evaluated by the method described by Tsung-Hu *et al.* (2010) [12] using 15 female rats (180-200g). Following a 24 h fast, laparotomy was performed under light ether anesthesia and the pylorus of each rat was tied followed by the closure of the abdominal incisions. The stomachs were removed 6 hours later and the stomach contents collected, centrifuged at 2000 rpm for 10 minutes and divided into four batches of beakers. Ninety milliliters of NaCl solution (0.9%), 90 mL of sodium bicarbonate, 25 mL of gastric juice and 90 mL of extract were placed in beakers and the pH was immediately measured at 25 °C. The temperature was then raised to 37 °C using a water bath and the pH was measured again immediately, and four hours later. Likewise, 90 mL of NaCl solutions (0.9%), 90 mL of sodium bicarbonate and 90 mL of extract were again placed in beakers. 25 mL of gastric juice were added to each beaker and the pH of the mixtures was measured at 25 °C and at 37 °C.

2.6 Evaluating the cytoprotective capacity of the extract against indomethacin in rats pre-treated with L-NAME

The effect of L-NAME pre-treatment on the protective power of the ethanolic extract of *E. praetermissa* on gastric lesions induced with indomethacin in rats fasted for 24 hours was evaluated using a modification of the method described by Mahmood *et al*. (2005) [13].

Five groups of five rats were treated as follows

1. Group I (normal rats): the animals were pre-treated with physiological solution (1 mL/ 200 g of 0.9% NaCl (*ip*)), and received by gavage, 30 min later, 1 mL/200 g of distilled water, and 60 min later laparotomy was performed under light ether anesthesia and the pylorus of each rat was tied followed by the closure of the abdominal incisions;
2. Group II (negative control 1): the animals receive similar treatment to group I rats, but immediately after the suturing, they were injected (*ip)* with indomethacin (30 mg/kg);
3. Group III (negative control 2): the animals were pre-treated with L-NAME (50 mg/kg, *ip*), then received by gavage, 30 min later, 1 mL/200 g of distilled water and, 60 min later laparotomy was performed under light ether anesthesia and the pylorus of each rat was tied followed by the closure of the abdominal incisions. Immediately after suturing the animals received an *ip* injection of indomethacin (30 mg/kg);
4. Group IV (test group 1): the animals were pre-treated with physiological solution, then received by gavage, 30 min later, 500 mg/kg of ethanolic extract of *E. praetermissa* and, 60 min later, laparotomy was performed under light ether anesthesia and the pylorus of

each rat was tied followed by the closure of the abdominal incisions. Immediately after the suturing, the animals received *ip*injection of indomethacin (30 mg/kg);

5. Group V (test 2): the animals were treated in the same way as for group III except that following pre-treatment with L-NAME (50 mg/kg, *ip*), they received 500 mg/kg of ethanolic extract of *E. praetermissa*in place of distilled water.

2.8 Statistical analysis

Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Dunnett's test. The values were expressed as mean ± SEM. *P*-values < 0.05 were considered as statisticallysignificant.

3. Results

3.1 Healing effect of ethanolic extract of *E. praetermissa* on chronic acetic acid-induced gastric ulcers in rats.

The extract (250 and 500 mg/kg) significantly (*p<0.001*) healed chronic ulcers following 10 days of treatment compared with controls, with very significant reduction of ulcer craters at 500 mg/kg (healing rate, 97.67%). Auto-healing in vehicle controls was accompanied by an increased mucus production (69.28 mg) compared with the 4-day controls (62.20 mg), as well as in extract-treated groups up to 85.92 mg for the 500 mg/kg dose (Table 1).

Table 1I: Healing effect ofethanolic extract of *E. praetermissa*on chronic acetic acid-induced gastric ulcers in rats.

Groups	Dose (mg/kg)	Ulcer index	% ulcerated area	% healing	Mucus production (mg)
Control 1	-	47. 20 ± 3,36	6.99	-	62.20 ± 6.75
Control 2	-	23.60 ± 1.12	3.50	50	69.28 ± 6.26
E. praetermissa	250	3.00 ± 0.16***	0.44	93.64	75.56 ± 3.90
E. praetermissa	500	1.10 ± 0.10***	0.16	97.67	85.92 ± 6.21
Sucralfate	50	3.60 ± 0.24***	0.53	92.37	72.09 ± 11.47

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 relative to control 2; *p<0.05; ***p<0.001; The values are expressed as mean ± SEM.

Figure 1 shows histological sections with normal mucosa (Photo 1), exulceration (transverse control group 1); hyperplasia of the para-ulcerated glands (longitudinal control 2) indicating normal healing process with absence of exulceration and fibrosis. In extract-treated rats there was gradual normalization of stomach tissue resulting in the absence of crater and significant progression of healing at 250 mg/kg. The 500 mg/kg dose of extract and sucralfate showed more marked healing.

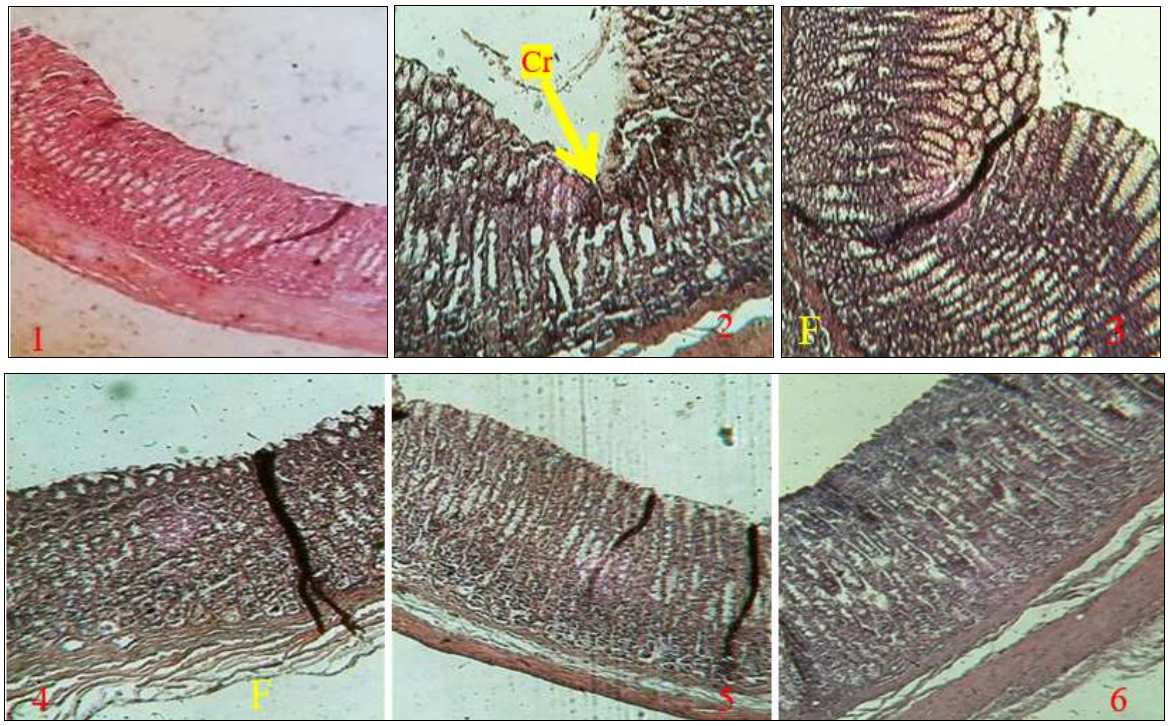

Fig 1: Histological presentation of the chronic acetic acid-induced ulcers.

(1): Normal control group (untreated, non-ulcerated rats that received distilled water for 10 days).(2): Control 1 (ulcerated rats sacrificed 4 days after acetic acid ulcer induction). (3): Control 2 (ulcerated rats given vehicle for 10 days following ulcer induction).(4) and (5) Groups treated with extract of E. praetermissa respectively at 250 mg/kg, 500 mg/kg.(6):group 5 ulcerated rats given 50 mg/kg sucralfate (reference drug). Cr: ulcer crater. F: fibrosis. (→ crater indication).

3.2 Healing effect of the extract of *E. praetermissa* on chronic Ethanol/Aspirin-induced gastric ulcers in rats

Table 2 shows that ulcer index reduced from 6.55 in the 5-day controls to 5.40 in the vehicle controls, indicating auto-healing of 17.55%. Following two weeks treatment with extract, healing rate was 100% at 500 mg/kg dose, with significantly higher levels of mucus production (140.0 mg) compared to the vehicle control 2 (121.20 mg).).

Table 2: Healing effect of the extract of *E. praetermissa* on Ethanol/Aspirin-induced chronic gastric ulcers in rats.

Groups	Dose (mg/kg)	Ulcer index	% ulcerated area	% healing	Mucus production (mg)
Control 1	-	6.55 ±0.38	0.97	-	105.6 ± 1.72
Control 2	-	5.40 ± 0.10	0.80	17,55	121.20 ± 3.37
E. praetermissa	250	0.00 ± 0.00***	0.00	100	127.8 ± 5.44
E. praetermissa	500	0.00 ± 0.00***	0.00	100	140.0 ± 3.81 *
Ranitidine	50	4.04 ± 0.38**	0.60	38.32	133.40 ± 5.56

Control 1 (ulcerated rats sacrificed 5 days after ethanol/aspirin induction); Control 2 (ulcerated rats given vehicle for 10 days following ulcer induction). Statistically different relative to control 2; *p<0.05; **p<0.01; ***p<0.001. Values are expressed as mean ± SEM

In Figure 2, Photo (1) shows the mucous membrane of a normal rat. Transverse group rats showed ulcer craters that descended across the musclaris mucosae to reach the muscle layer. Rats from the longitudinal group exhibited a self-healing process with reduction of the ulcer crater and the existence of para-ulcerated glandular hyperplasia. In rats treated with the extract at a dose of 250 mg/kg, the absence of the crater was noted with significant re-epithelialization. At the dose of 500 mg/kg, recovery was almost complete with significant re-epithelialization. The rats treated with ranitidine showed an advanced healing process with incomplete re-epithelialization but with persistence of the ulcer crater.

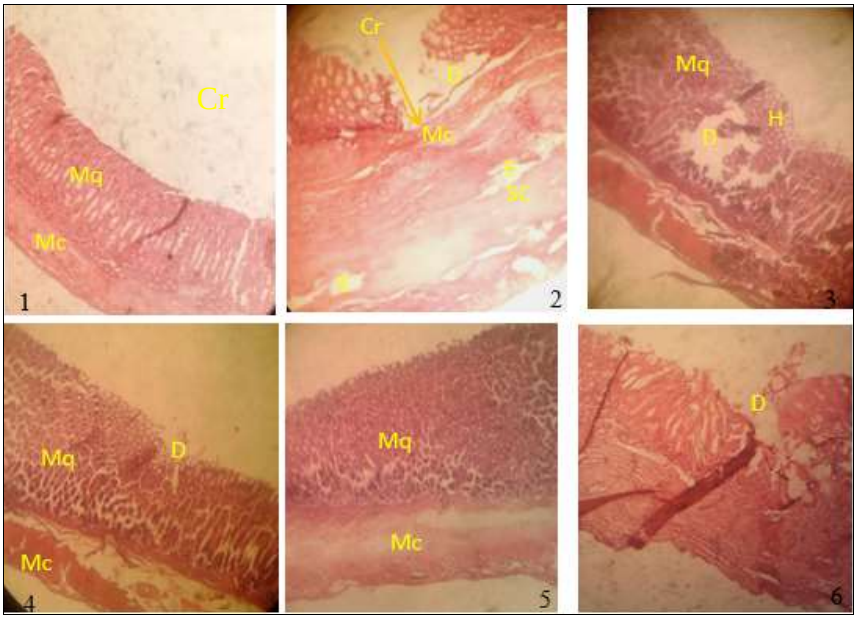

Fig 2: Histological presentation of the chronic Ethanol/Aspirin-induced gastric ulcer.

(1): Normal control group (untreated, non-ulcerated rats that received distilled water for 10 days). (2): Control 1 (ulcerated rats sacrificed 5 days after ethanol/aspirin induction). (3): Control 2 (ulcerated rats given vehicle for 10 days following ulcer induction). (4) and (5) Groups treated with extract of E. praetermissa respectively at 250 mg/kg, 500 mg/kg. (6): group 5 ulcerated rats given 50 mg/kg of Ranitidine (reference drug). Cr: ulcer crater; D: destruction; E: edema; Mq: mucosa; Mc: Muscle layer; Sc: sclerosis. (→ulcer indication).

3.3 *In vitro* antacid activity of *E. praetermissa* ethanolic extract

The initial pH values of sodium bicarbonate solutions, gastric juice and ethanolic extract of *E. praetermissa* were, respectively, 9.28; 2.90 and 4.09 at a temperature of 25 °C. When the different solutions were maintained at a temperature of 37 °C, a slight acidification of the gastric juice and a slight basification of the extract were observed. Sodium bicarbonate increased the pH of gastric juice from 2.90 to 8.75 at 25 °C and from 2.06 to 8.20 at 37 °C. *E. praetermissa* extract increased the pH of gastric juice only slightly from 2.90 to 3.15 at 25 °C. This increase in pH was much more marked at 37 °C, the pH of gastric juice increasing from 2.06 to 4.07. The duration of incubation (4h) did not significantly change the pH of the different solutions at both temperatures (Table 3).

Table 3: The pH values of the different solutions before and after incubation at temperatures of 25 °C and 37 °C

Solutions	pH values at 25 ° C		pH values at 37 °C	
	Immediately after centrifuging	4 Later	Immediately after centrifuging	4 Later
Gastric juice	2.90 ± 0.07	2.57 ± 0.24	2.06 ± 0.05	2.06 ± 0.09
NaCl (0,9 %)	7.08 ± 0.05	7.09 ± 0.08	7.30 ± 0.13	7.29 ± 0.25
$NaHCO_3$ (100 mg/mL)	9.28 ± 0.10	9.09 ± 0.22	9.24 ± 0.04	9.09 ± 0.06
E. praetermissa (100 mg/mL)	4.09 ± 0.27	4.09 ± 0.07	5.14 ± 0.22 $^{\mu\mu}$	5.20 ± 0.41 $^{\mu\mu}$
NaCl + gastricjuice	2.60 ± 0.04	2.61± 0.16	2.05 ± 0.05	2.04 ± 0.07
$NaHCO_2$ + gastricjuice	8.75 ± 0.30***	8.63 ± 0.21	8.20 ± 0.13***	8.20 ± 0.13***
E. praetermissa+ gastricjuice	3.15 ± 0.46	3.39 ± 0.02	4.07 ± 0.38**	4.05 ± 0.32**

The values represent the means ± ESM (number of samples = 5); ** p<0.01 and *** p<0.001: statistically significant difference compared to the negative control (gastric juice); $^{\mu\mu}p$<0.01: statistically significant difference compared to the same solution at a temperature of 25 °C.

3.4 Effects of ethanolic *E. praetermissa* extract on indomethacin-induced gastric injury in rats pre-treated with L-NAME

Ulcer index was 4.30 with pylorus ligation alone, 13.30 when pylorus ligation was associated with indomethacin treatment, and further increased to 25.20 following pretreatment with L-NAME. Administration of extract prior to pylorus ligation/indomethacin reduced ulcer index from 13.30 to 7.70, and extract administration after pre-treatment with L-NAME reduced ulcer index from 25.20 to 9.70. Indomethacin did not significantly reduce the nitric oxide content of mucus (0.030 µg/mg of mucus), but pre-treatment with L-NAME reduced the values by 50% (0.015 µg/mg of mucus). However, extract administration restored gastric mucus nitric oxide levels to above normal values (0.049-0.056 µg/mg of mucus). The beneficial effects of extract on ulcer index and nitric oxide levels were associated with significant increases in gastric mucus production.

Table 4: Effects of ethanolicextract of *E. praetermissa* on indomethacin-induced gastric injury in rats pre-treated with L-NAME

Groups	Ulcer index (IU)	% S.U	% I	Mucus production (mg)	Nitric oxide production (µg/mg of mucus)
Normal rats (Dist. H_2O/pylorus ligation)	4.30 ±.75**	0.64	-	29.40 ± 2.38*****	0.032 ± 0.007
Control 1 (Dist. H_2O/pylorus ligation/indometacin)	13.30 ± 0.66 §§§	1.97	-	20.80 ± 2.40	0.030 ± 0.002
Control 2 (L-NAME/pylorus ligation/indomethacin)	25.20 ± 2.97***	3.73	-	31.20 ± 1.77*****	0.015 ± 0.0007*****
Extract/ pylorus ligature/indomethacin	7.70 ± 1.06 *	1.14	42.11	68.10 ± 1.99*******	0.056 ± 0.005******
L-NAME /extract/ pylorus-ligation /indomethacin	9.70 ± 0.97 §§§	1.43	27.07	63.60 ± 2.50*******	0.049 ± 0.002*****

N: number of rats = 5; % S.U = percentage of ulcerated area; % I = percent inhibition relative to negative control 1 and negative control 2, respectively; values represent means ± ESM; * $p<0.05$; ** p <0.01 and *** $p<0.001$: statistically significant compared to control 1. §§§ $p<0.001$: statistically significant compared to control 2.

4. Discussion

The acetic acid-induced chronic ulcer model easily produces characteristic circular and deep ulcers in the stomach pathologically and therapeutically similar to human gastric ulcers. [11]. These ulcers are produced mainly due to the increased secretion of acid by the parietal cells that cause mucosal necrosis [15]. The increased gastric secretion causes necrosis, transforming superficial lesions into deeper wounds [16]. Tissue necrosis attracts leukocytes (polymorphonuclear neutrophils and macrophages) which phagocytize necrotic tissue and release pro-inflammatory cytokines and growth factors that activate fibroblasts, endothelial cells and epithelial cells. This activation is at the origin of the formation of granulation tissue as part of the healing process [17]. Ulcerated rats treated for ten days with the extract at doses of 250 to 500 mg/kg showed a percent cure of 93.64 and 97.67 accompanied by increased mucus secretion which protects the ulcer crater against the necrotic action of gastric acid [18]. The mucus strengthens the mucobicarbonate barrier thus enhancing the healing process.

The combined aggressive actions of ethanol and aspirin produce chronic gastric ulcers. Alcohol stimulates acid secretion, reduces blood flow leading to gastric damage [19], and reduces bicarbonate and mucus secretion. Aspirin is an NSAID that interferes with prostaglandin synthesis (inhibiting cyclo-oxygenases), by increasing acid secretion and backscattering of H^+ ions [20], thus reducing the secretion of bicarbonates and mucus, as well as blood flow to the mucous membrane [21]. These events reduce cytoprotection of the mucosa leading to ulcerations [22], and justify severity of ulceration observed in the cross-sectional control. The self-healing observed is due to the stimulation of the secretion of growth factors by the mucosa adjacent to the ulcer site, leading to gradual reconstruction of the damaged mucosa [23].The extract of *E. praetermissa* (250 and 500 mg/kg) significantly reduced ulcer index, with total disappearance of the ulcer crater and significant increase mucus secretion.

Histological analysis of chronic ulcers caused by the combination of ethanol and aspirin revealed severe inflammation in the cross-sectional controls. The increased vasodilation and permeability of the wall of the small vessels in the injured area are at the origin of the passage of exudate containing water and plasma proteins into the interstitial connective tissue [24]. Normalization of the tissue in rats treated with the ethanolic extract of *E. praetermissa* results in re-epithelialization of the mucosa, which shows that the extract would accelerate the healing of the ulcer and promote regeneration of the gastric mucosa largely thanks to the epithelial cell layer which produces mucus, bicarbonate, and other components of the muco-bicarbonate barrier [25]. Outer epithelial cells are also tightly connected by tight junctions, forming a continuous barrier, which prevents re-diffusion of acid and pepsin [26].

The pathophysiology of NSAIDs-induced gastric ulcers is associated with inhibition of cyclooxygenase and cyclooxygenase-independent mechanisms, which mainly result from their direct actions on the gastric mucosa [27]. The delay in ulcer healing due to heavy consumption of NSAIDs is associated with complications such as hemorrhages which can be fatal. COX-2 inhibitors are a specific group of NSAIDs that block the isoform of cyclooxygenase antibodies involved in inflammation. COX-2 stimulates cell proliferation and decreases the level of leukotrienes [28]. The results of this study showed that the extract healed chronic gastric ulcers potentiated by aspirin despite its irreversible action on COX-1 and COX-2. This suggests that the ethanolic extract of *E. praetermissa* may act in a way other than COX stimulation.

Antacids heal stomach ulcers by removing stomach acid or neutralizing the acidity. This action elevates gastric pH and thus provides tremendous relief from the symptoms of gastric hyperacidity [29]. Most commercial antacids are often unacceptable due to side effects, particularly constipation, diarrhea and toxicity [30], and significant interactions can occur with antibiotics (tetracycline), and iron sulfate [31]. The results if the present experiment suggest that temperature has no significant effect on the pH, despite the slight acidification of the gastric juice and a slight basification of the extract at 37 °C. When sodium bicarbonate and extract solutions were incubated with gastric juice, they increased the pH of the juice. However, it is known that excessive use of sodium bicarbonate can modify acid-base metabolism, and consequently increase blood pressure and cause problems, in particular for hypertensive or heart failure patients. The mineralogical composition of the extract of *E. praetermissa*

(with slightly basic pH, 4.09-5.14) may be responsible for the increase in the pH of gastric juice from 2.06 to 4.07 after incubation. The flavonoids present in the extract comprise a series of biologically active compounds which are ubiquitous in plants used in traditional medicine for the treatment of gastric ulcers [32]. The extract can inactivate the activity of pepsin because it increased the pH of gastric juice at 37 °C to 4.07. However, at pH between 4 and 6, pepsin is still stable but inactive (pepsinogen) [16]. The increase in pH of gastric juice by the extract was greatest at 37 °C.

The present study highlighted the importance of mucus NO in protecting against gastric ulcers induced by indomethacin injection. NO is synthesized by endothelial cells in the gastric mucosa primarily from L-arginine under the action of constitutive NO synthetase [33]. L-analogs of L-arginine such as L-NAME are competitive substrates for NO synthetase that inhibit NO synthesis. L-NAME has been shown to aggravate ulcer formation and *E. praetermissa* extract prevented indomethacin-induced gastric ulcer formation. These effects correlate well with the nitrite level of the mucus and the weight of the mucus suggesting that the extract prevents gastric ulcers by stimulating endothelial cells that synthesize and release NO, since NO and PGI_2 oppose the detrimental action of vasoconstrictors such as thromboxane A_2, leukotriene C_4, and endothelin on the gastric mucosa [34]. Apart from maintaining gastric blood flow, NO protects the gastrointestinal tract by inhibiting gastric acid secretion by parietal cells, stimulating mucus and bicarbonate secretion and accelerating angiogenesis *in vivo* and *in vitro* [35].

5. Conclusion

The cytoprotective and curative activity of the ethanolicextract of *E. praetermissa* could be due to a combination of its ability to increase mucus secretion by a mechanism similar to that of endogenous prostaglandins, to neutralize gastric acidity and to stimulate endothelial cells which synthesize and release nitric oxide.

6. Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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