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PREVENTIVE AND CURATIVE PROPERTIES OF AQUEOUS AND METHANOLIC EXTRACTS OF *DISTEMONANTHUS BENTHAMIANUS* STERM-BARK ON ACUTE AND CHRONIC GASTRIC ULCERS IN MALE RATS

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ABSTRACT

Background: A number of studies have been carried out to determine the efficacy of herbal medicines in the treatment of gastric ulcer. The present study was undertaken to evaluate the preventive and curative properties of aqueous and methanolic extracts of *Distemonanthus benthamianus* stem-bark on experimentally induced gastric ulcers in male rats. **Methods:** The aqueous and methanolic extracts of *Distemonanthus benthamianus* were administered at the doses 125, 250 and 500 mg/kg to evaluate their effects on gastric ulcer induced models with ethanol, indomethacin and acetic acid in Wistar male adult rats, weighing between 180 and 220g. Histopathological examination and nitric oxide level were performed in sub-chronic acetic-acid induced gastric ulcers. **Results:** Aqueous extract at dose 500 mg/kg produced 99.98, 99.23, 98.74 and 100% inhibition in gastric ulcerations induced respectively by ethanol, indomethacin and acetic acid; while methanol extract at the same dose produced 99.94, 99.63, 100 and 100% inhibition on ulcerations induced respectively by the above ulcerogenic reagents. Histopathology analysis showed a complete regeneration of stomach layers in animals treated with both extracts at doses 250 and 500 mg/kg. Results also revealed a significant ($p<0.001$) increase in nitric oxide (NO) level with methanol extract. Furthermore, a significant ($p<0.001$) increase in mucus production was recorded in all evaluated gastric ulcer induced models. **Conclusions:** Study revealed that preventive and curative properties of aqueous and methanolic extracts of *Distemonanthus benthamianus* stem-bark could be due to cytoprotective mechanisms, increased mucus production, and also release of NO.

KEYWORDS: Gastric Ulcers, Ulcerogenic reagents, *Distemonanthus benthamianus* extracts, curative and preventive.

INTRODUCTION

Gastric ulcer is a gastrointestinal disease which can be defined as a rupture that extends through the *Muscularis mucosa* into the submucosa or deeper.[1] Some of the main aggressive factors that can lead to ulcer are gastric acid, pepsin, bile salts, abnormal motility, alcohol, non-steroidal anti-inflammatory drugs (NSAID) and *Helicobacter pylori* infection.[2] Several factors can protect the stomach from ulcer formation; such as mucus secretion, prostaglandin synthesis, bicarbonate production and normal tissue microcirculation,[3,4] thus reducing gastric acid production and increasing gastric mucosal protection have been the major strategies proposed for the prevention of peptic ulcer disease.[5] A number of synthetic drugs such as proton pump inhibitors (Omeprazole), H_2 receptor antagonists (Ranitidine) and other non-steroidal anti-inflammatory drugs are available to treat ulcers, leading to a decrease in mortality and morbidity rates. However, they are not always completely effective and some of them produce many adverse effects when compared to herbal medicines.[6] In this context, the use of medicinal plants for the prevention and treatment of different pathologies is in continuous expansion worldwide.[7] Medicinal plants, spices, vegetables, and crude drug substances are considered to be a potential source to fight against various diseases including gastric ulcer.[8,9] Therefore, as part of research activities in validation of medicinal plants used in traditional medicine in Cameroon, *Distemonanthus benthamianus* (Fabaceae) was chosen. This plant is one of the perennial trees of the evergreen, semi-deciduous and secondary forest. It appears mainly in Cameroon, Ghana and Nigeria,[10] and in Cameroon this plant is used to treat urogenital tract infection while in traditional African medicine it is used in treating bacterial, fungal and viral infections.[11] Despite the use of its stem-bark as antiulcer agents in folk medicine, there are none or few scientific studies to explain its

action mechanisms which make the plant be considered as anti-ulcer. Therefore, the present study was aimed at investigating the preventive and curative properties of aqueous and methanolic extracts from *Distemonanthus benthamianus* stem-bark on induced gastric ulcers in male rats.

MATERIALS AND METHODS

2.1. Collection of plant material

Fresh stem-bark of *Distemonanthus benthamianus* whas collected at Suza (Littoral region, Cameroon) on April 2014, and authenticated at the National Herbarium in Yaounde (Cameroon) through a comparison with the voucher specimen No.TN 275. Those stem-bark were further scrapped, chopped, shade dried and powdered.

2.2. Preparation of plant extract

The aqueous extract was prepared by maceration of 300 g of powder of *Distemonanthus benthamianus* stem bark in 1.5l of distilled water for 72h. After filtration with filter paper (Whatman no 1), the filtrate was concentrated in a Selecta-25102 oven at 35°C, to give 20 g of the aqueous extract corresponding to an extraction yield of 6.7% (w/w).

The other portion of stem bark powder (300 g) was macerated in 3l of methanol for 72h and the solvent removed from the extract under reduced pressure, using a Büchi (R-124) rotary evaporator at 65°C. This gave 22 g of the methanol extract, corresponding to a yield of 7.3% (w/w).

2.3. Chemicals and drugs

DMSO (Dimethyl sulfoxyde), ethanol, indomethacin, acetic acid, and Griess reagent were obtained from Sigma Chemicals. Omeprazole, Aluminium hydroxide plus magnesium hydroxide (Maalox), Ranitidine and Misoprostol were purchased from pharmacy. All chemicals and reagents used were of analytical grade.

2.4. Animals

Wistar albino male rats weighing between 180 - 200 g were used. They were bred in the Animal House of the Department of Animal Biology, University of Dschang, Cameroon under natural room conditions. Animals were fed with a standard diet and received water *ad libitum*. Prior to experimental protocol, the rats were acclimatized for 48h to laboratory conditions for minimizing any nonspecific stress. Experimental protocols used in this study were approved by the laboratory committee (Laboratory of Animal Physiology and Phytopharmacology, Department of Animal Biology, Faculty of Science, University of Dschang-Cameroon) according to the standard ethical guidelines for Laboratory animal use and care as described in the European Community guidelines; EEC Directive 86/609/EEC, of the 24th November 1986.[12]

2.5. Anti-ulcer studies

2.5.1. Ethanol-Induced Gastric Mucosal Lesions

Gastric mucosal lesions were induced in male rats using the ethanol method as described by.[13] The animals were fasted for 24 h and divided into 9 groups consisting of 6 rats per group. Groups 1 and 2 which represent the negative control groups, received distilled water and DMSO 3% respectively. Group 3, representing the positive control groups received 20 mg/kg body weight of omeprazole. Groups 4 to 6 received aqueous extract at doses of 125, 250 and 500 mg/kg respectively. Groups 7 to 9 received methanolic extract at doses of 125, 250 and 500 mg/kg. All test substances were orally administered at 1ml/100 g b.w. One hour after the administration of ethanol, each animal was sacrificed by cervical dislocation with a gastric cannula, stomach was removed and opened along the greater curvate, washed and the surface area of each lesion was measured and scored as described by.[14] Ulcer index for each rat was recorded as the mean ulcer score (0: no ulcer; 1: $US \leq 0.5mm^2$; 2: $0.5mm^2 < US \leq 2.5mm^2$; 3: $2.5mm^2 < US \leq 5mm^2$; 4: $5mm^2 < US \leq 10mm^2$; 5: $10mm^2 < US \leq 15mm^2$; 6: $15mm^2 < US \leq 20mm^2$; 7: $20mm^2 < US \leq 25mm^2$; 8: $25mm^2 < US \leq 30mm^2$; 9: $30mm^2 < US \leq 35mm^2$; 10:$US > 35mm^2$). The percentage ulcerated surface was calculated as the total area covered by all lesions expressed as a proportion of the total corpus mucosal surface area, gastric mucus of each stomach was collected and weighed. The percentage of inhibition (%I) was calculated using the following formula:

$\%I = (USc - USt) \times 100/USc$ .Where USc = Ulcer surface area of control and USt = Ulcer surface area of test animal.

2.5.2. Indomethacin-induced ulcers (Preventive Test)

The method described by[15] was adopted for this study. 9 groups of 6 animals each were used. Groups 1 and 2 reveived distilled water and DMSO 3% (1ml/100g b.w) respectively. Group 3 considered as positive control were pretreated with 100µg/kg of Misoprostol. Groups 4 to 6 received 1ml/100g body weight aqueous extract at doses of 125, 250 and 500mg/kg respectively and groups 7 to 9 received 1 ml/100g body weight methanolic extract at doses of 125, 250 and 500mg/kg. One hour after drug administration, each animal received 30 mg/kg body weight of Indomethacin. All treatments were orally administered. The animals were sacrificed 5 hours after treatment by cervical dislocation, the stomach was excised, rinsed and examined for ulceration. Ulcerations produced were scored, ulcer index and the percentage of inhibition were estimated as previously described. Mucus covering the gastric wall of each rat was collected and weighed.

2.5.3. Indomethacin-induced ulcers (Curative Test)

Indomethacin-induced model was used as described by.[16] Thus 60 animals fasted for 24 h received indomethacin (5 mg/kg) for five days. They were then divided into 10 groups (n= 6) and treated once daily for

another five days with the respective test solutions. Groups 1 and 2 (negative control) received distilled water and DMSO 3%, group 3 and 4 (positive control) received 50 mg/kg Maalox and 100 µg/kg Misoprostol; group 5, 6, 7 received 125, 250 and 500 mg/kg of aqueous extract respectively and groups 8, 9, 10 received methanolic extract at the same above doses.

After oral administration of test solutions, the rats were sacrificed on the sixth day, the stomachs were removed and opened along the greater curvate and washed. Gastric lesions were observed and the ulcer index was determined as follows:[17] ***1*** *(ulcerated area: 1-6 mm^2),* ***2*** *(ulcerated area: 7-12 mm^2),* ***3*** *(ulcerated area: 13-18 mm^2),* **4** *(ulcerated area: 19-24 mm^2),* ***5*** *(ulcerated area > 24 mm^2).* The gastric mucus of each stomach was collected and weighed.

2.5.4. Acetic acid-induced gastric ulcers

The acetic acid-induced gastric ulcers model was performed as described by.[18] Sixty-six male Wistar rats fasted for 24 h were used in this experiment. Under anesthesia resulting from a Diazepam (5 mg/ml)/Ketamine (50 mg/ml) (2:1 v/v) mixture, a laparotomy was done on all animals through a midline epigastric incision and six other animals were used as normal control group. After exposing the stomach, 0.05 ml of 30% (v/v) acetic acid solution was injected into the subserosal layer, of the anterior glandular part. It was therefore bathed with saline, to avoid adherence to external surface of ulcerated region and opened abdomen region was then sutured. One day after the induction animals were orally treated once a day for 14 consecutive days with; distilled water (normal control group); distilled water or DMSO 3% (negative control groups); Ranitidine (50 mg/kg) and Maalox (50 mg/kg) served as positive control groups; aqueous and methanol extracts at doses of 125, 250 and 500 mg/kg.

On the 15th day, all animals were anesthetized, blood was therefore collected by abdominal artery catheterization, centrifuged (3000 rpm/ 15min) and collected plasma was preserved at -18°C for NO levels evaluation. Furtheremore, stomach was removed for evaluating ulcerated area, mucus weight, tissue nitric oxide level dosage and histological assessement.

2.6. Histological assessment

One sample of the removed stomachs was preserved in 10% formalin solution, followed by tissue dehydration with alcohol and xylene. Each sample was embedded in paraffin wax, sectioned at 5 µm in slides prior for staining. Haematoxylin and eosin stain was used and the slides were examined under light microscope using 40x lenses.

2.7. Nitric oxide dosage

After acetic acid induction, the heparinized blood and the homogenized stomach of the rats were used to measure the Nitric oxide level measured in accordance with the method described by.[19] Thus NO content was quantified by measuring nitrite/nitrate concentration using Griess assay, and sodium nitrite was used as standard. Briefly, gastric homogenates were centrifuged at 3500 rpm for 25 min and the supernatant obtained, associated to the previously collected plasma was each supplemented with Griess reagent and absorbance was measured at 540 nm. Results were expressed as µmol/g tissue.

2.8. Statistical analysis

For statistical analysis of data, multiple comparison tests were carried out using One-way analysis of variance (ANOVA) followed by Tukey post-test. Results were expressed as mean ± standard deviation (s.d) and statistical significance was acceptable at $p< 0.05$, $p< 0.01$ and $p< 0.001$. The GraphPad 5.0 software progam was used.

RESULTS

3.1. Ethanol-Induced Gastric Mucosal Lesions

Oral administration of 95% ethanol provoked a longitudinal gastric ulcer in the stomach glandular part, as observed in rats from the negative control group, which presented a surface area of 23.80±14.48 and 131.20±6.78 mm² when treated respectively with distilled water and DMSO3% (table 1 and figure 1). Aqueous extract of *Distemonanthus benthamianus* produced a dose-dependent inhibition of 74.16%, 87.34% and 99.98% (for dose 125, 250 and 500 mg/kg respectively) and corresponding to a significant ($p<0.001$) ulcerated area reduction of 35.35 ± 0.86, 17.32 ± 0.35 and 0.03 ± 0.03 mm² for the same Preceding doses respectively when compared to that observed in distilled water treated negative control group. Methanolic extract has presented the best effect, by significantly ($p<0.001$) inhibiting ulcerations with percentages of 88.81%, 99.97% and 99.94% (for dose 125, 250 and 500 mg/kg respectively), corresponding to ulcerated area of 14 .68 ± 1.43, 0.03 ± 0.03 and 0.08 ± 0.08 mm², respectively for the same doses.

Also, the mean ulcer index score has significantly ($p<0.001$) decreased from 6.12 ± 0.49 in distillled water treated negative control rats group to 3.88 ± 0.22, 2.17 ± 0.16 and 0.00 ± 0.00 in animals respectively treated with doses 125, 250 and 500 mg/kg and from 5.20 ± 0.49 in DMSO3% treated negative control group to 3.48 ± 0.36, 0.00 ± 0.00 and 0.00 ± 0.00 in animals which received respectively the same doses as mentioned with the aqueous extract.

Table 1: Preventive effects of aqueous and methanolic extracts of *Distemonanthus benthamianus* stem bark on ethanol-induced gastric lesions in rats.

Treatement	Dose (mg/kg)	n	US (mm^2)	%US	UI	%I	Mucus Weight (mg)
Distilled water	1ml/100g b.w	6	236.80 ± 14.48	24.57 ± 3.60	6.12 ± 0.49	/	58.83 ± 1.78
DMSO (3%)		6	131.20 ± 6.78	13.14±1.20	5.20 ± 0.49	/	33.50 ± 1.38
Omeprazole	20	6	57.83 ± 0.26^{C}	5.81 ± 0.56^{C}	3.70 ± 0.25^{Ca}	57.72	34.17 ± 0.87^{c}
Aqueous extract of *D. benthamianus*	125	6	35.35 ± 0.86^{C}	3.59 ± 0.28^{C}	3.88 ± 0.22^{C}	74.16	46.50 ± 0.72$^{C\gamma}$
	250	6	17.32 ± 0.35$^{C\beta}$	2.12 ± 0.15$^{C\beta}$	2.17 ± 0.16$^{C\beta}$	87.34	55.00 ± 1.10$^{\gamma}$
	500	6	0.03 ± 0.03$^{C\gamma}$	0.00 ± 0.00$^{C\gamma}$	0.00 ± 0.00$^{C\gamma}$	99.98	73.67 ± 0.84$^{C\gamma}$
Methanol extract of *D. benthamianus*	125	6	14 .68 ± 1.43$^{C\gamma}$	1.55 ± 0.23$^{C\gamma}$	3.48 ± 0.36^{b}	88.81	32.83 ± 0.85
	250	6	0.03 ± 0.03$^{C\gamma}$	0.00 ± 0.00$^{C\gamma}$	0.00 ± 0.00$^{C\gamma}$	99.97	46.17 ± 0.79$^{C\gamma}$
	500	6	0.08 ± 0.08$^{C\gamma}$	0.01 ± 0.01$^{C\gamma}$	0.00 ± 0.00$^{C\gamma}$	99.94	65.33 ± 1.15$^{C\gamma}$

n = number of animals per group, US=Ulcerated area, %US= Ulcerate area percentages, UI= Ulcer index, %I= inhibition percentage, $^{a}p<0.05$; $^{b}p<0.01$; $^{c}p<0.001$: significant when compared to negatives controls groups (distilled water and DMSO 3%). $^{\beta}p<0.01$; $^{\gamma}p<0.001$: significant when compared to positive control group (Omeprazole), DMSO =Dimethyl sulfoxyde, b.w=body weight.

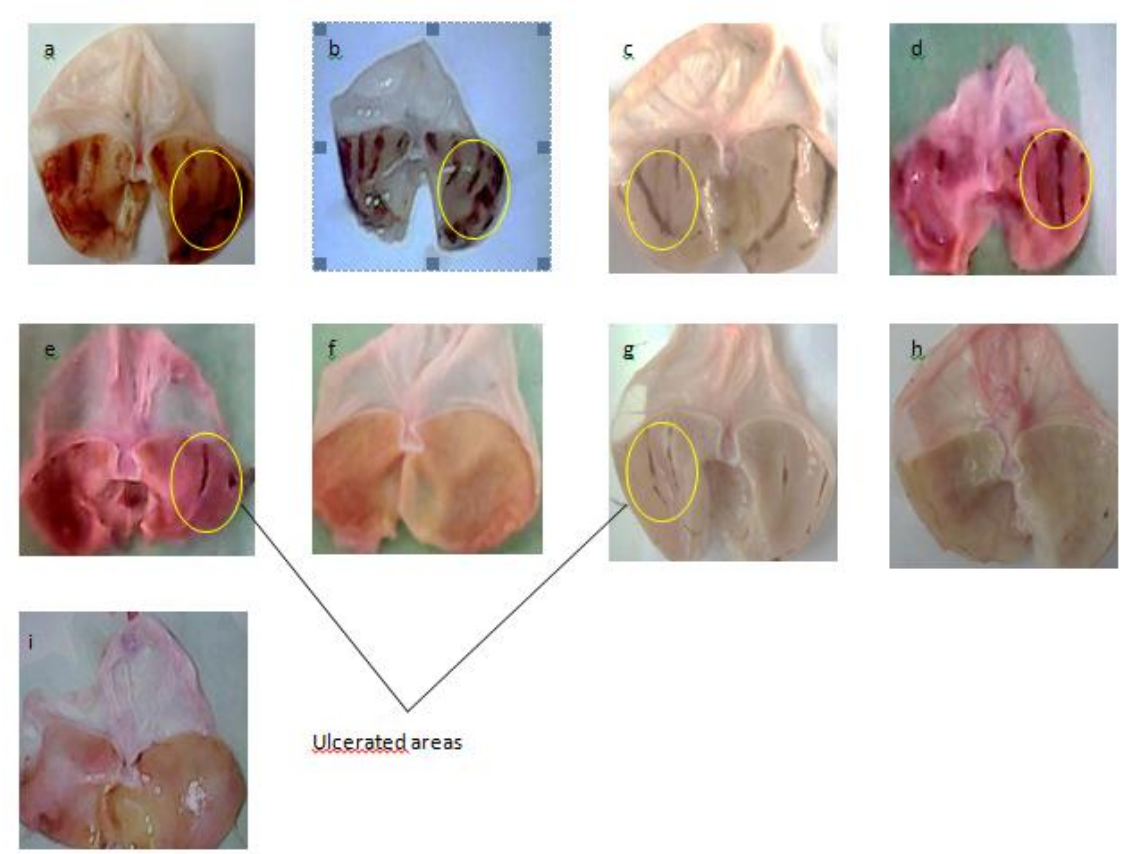


Fig. 1: Macroscopic appearance of gastric mucosa of rats.

(a) & (b) Distilled water and DMSO3% pre-treated rats: Severe lesions are seen in the gastric mucosa: ethanol produced extensive visible hemorrhagic necrosis of gastric mucosa. (c) Rats pre-treated with omeprazole (20 mg/kg): lesions in the gastric mucosa are partially comparable to those seen in negative control groups. (d) & (e) Rats pre-treated with aqueous extract at doses 125 and 250 mg/kg respectively: moderate lesions are seen in the gastric mucosa and lesion decrease as the dose increase. (f) Rats pre-treated with aqueous extract at dose 500 mg/kg: no lesions are seen, meaning that this dose extract has completely inhibited the gastric lesions induced by ethanol. (g), (h) & (i) Rats pre-treated with methanol extract at doses 125, 250 and 500 mg/kg: the lesions have reduced with the increased doses; while at 500 mg/kg no lesions are observed.

3.2. Indomethacin-induced ulcers (preventive test)

Table 2 and figure 2 Present the results obtained in preventive indomethacin-induced ulcers test. It revealed that in animals treated with doses 125, 250 and 500 mg/kg of aqueous extract, a significant ($p<0.001$) dose-

dependent inhibition of ulcerated area was recorded. Thus, those ulcerated areas have reduced to 14.53 ± 0.93, 3.65 ± 0.32 and 0.57 ± 0.14 mm² respectively, when compared to animal shaving that received distilled water (73.78 ± 0.65 mm²); this corresponding to an inhibition percentage of 80.30, 95.05 and 99.23% respectively. Similar results have been obtained with the methanolic extract which at the previously cited doses, has significantly ($p<0.001$) reduced the ulcerated areas to 21.75 ± 0.28, 3.01 ± 0.13 and 0.30 ± 0.19 mm² respectively when compared to animals having received DMSO3% (80.25 ± 1.78 mm²), and this corresponding to an inhibition percentage of 72.90, 96.25 and 99.63% respectively.

According to mucus weight, results showed that it has significantly ($p<0.001$) increased in animals having received doses 125, 250 and 500 mg/kg of aqueous extract with values respectively equal to 45.83 ± 1.11, 45.50 ± 1.18 and 53.50 ± 1.23 mg; when compared to negative control group animals, which received distilled water (22.50 ± 0.62 mg). Similarly, the methanolic extract has had the same effect, by significantly ($p<0.001$) increasing mucus weight in same doses treated animals, with values equal to 22.83 ± 0.95, 41.17 ± 0.75 and 40.50 ± 0.34mg respectively, when compared to animals which received DMSO3% (11.83 ± 0.91mg).

Table 2: Preventive effects of aqueous and methanolic extracts of *Distemonanthus benthamianus* stem bark on indomethacin-induced gastric lesions in rats.

Treatment	Dose (mg/kg)	n	US (mm^2)	%US	UI	%I	Mucus Weight (mg)
Distilled water	1ml/100g b.w	6	73.78 ± 0.65	11.21 ± 0.76	6.57 ± 0.37	/	22.50 ± 0.62
DMSO (3%)		6	80.25 ± 1.78	11.36 ± 0.25	6.50 ± 0.49	/	11.83 ± 0.91
Misoprostol	100µg/kg	6	27.95 ± 0.67^{c}	3.42 ± 0.21^{c}	5.20 ± 0.23^{a}	62.12	47.83 ± 1.72^{c}
Aqueous extract of *D.benthamianus*	125	6	$14.53 \pm 0.93^{c\gamma}$	$1.87 \pm 0.14^{c\alpha}$	4.58 ± 0.33^{c}	80.30	45.83 ± 1.11^{c}
	250	6	$3.65 \pm 0.32^{c\gamma}$	$0.46 \pm 0.02^{c\gamma}$	$3.00 \pm 0.00^{c\gamma}$	95.05	45.50 ± 1.18^{c}
	500	6	$0.57 \pm 0.14^{c\gamma}$	$0.08 \pm 0.02^{c\gamma}$	$1.17 \pm 0.31^{c\gamma}$	99.23	$53.50 \pm 1.23^{c\alpha}$
Methanol extract of *D.benthamianus*	125	6	$21.75 \pm 0.28^{c\gamma}$	2.94 ± 0.12^{c}	4.00 ± 0.13^{c}	72.90	22.83 ± 0.95^{c}
	250	6	$3.01 \pm 0.13^{c\gamma}$	$0.43 \pm 0.02^{c\gamma}$	$2.30 \pm 0.24^{c\gamma}$	96.24	41.17 ± 0.75^{c}
	500	6	$0.30 \pm 0.19^{c\gamma}$	$0.04 \pm 0.03^{c\gamma}$	$0.67 \pm 0.42^{c\gamma}$	99.63	40.50 ± 0.34^{c}

n = number of animals per group, US=Ulcerated area, %US= Ulcerate area percentages, UI= Ulcer index, %I= inhibition percentage, $^{a}p<0.05$; $^{b}p<0.01$; $^{c}p<0.001$: significant when compared to negatives controls groups (distilled water and DMSO3%). $^{\alpha}p<0.05$; $^{\beta}p<0.01$; $^{\gamma}p<0.001$: significant when compared to positive control group (Misoprostol), DMSO =Dimethyl sulfoxyde, b.w=body weight.

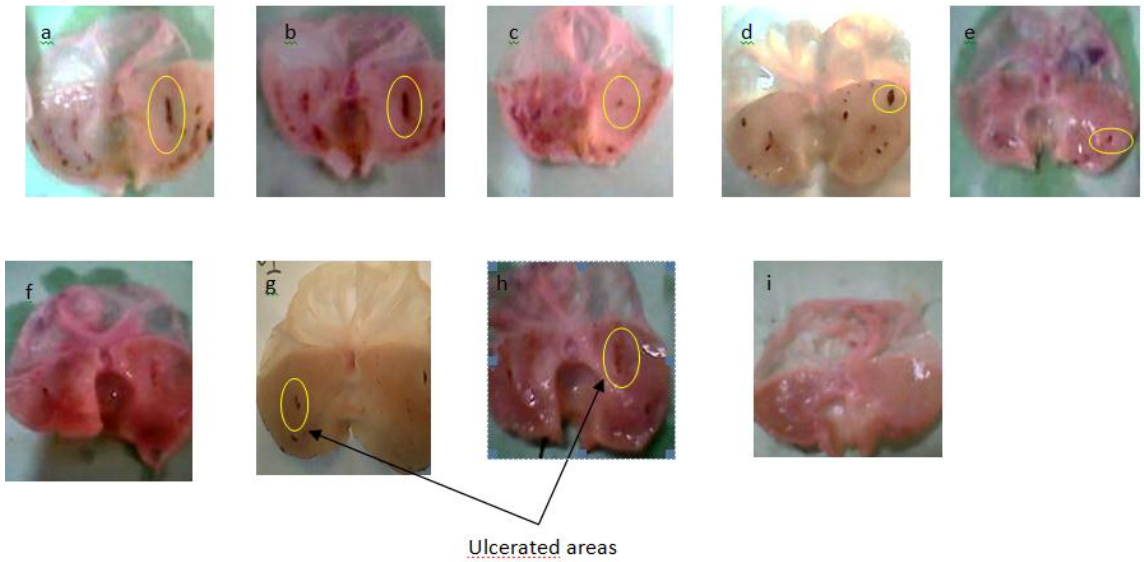


Fig. 2: Macroscopic appearance of the gastric mucosa of rats.

(a) & (b) Distilled water and DMSO3% pre-treated rats: severe lesions are seen in the gastric mucosa; Indomethacin produced hemorrhagic necrosis of gastric mucosa. (c) Rats pre-treated with Misoprostol (100µg/kg): lesions in the gastric mucosa are partially comparable to those seen in negative control groups. (d), (e) & (f) Rats pre-treated with aqueous extract at doses of 125, 250 and 500 mg/kg respectively: moderate lesions are seen in the gastric mucosa, and the lesions decreased when the dose increased; aqueous extract has reduced gastric lesions induced by indomethacin. (g), (h) & (i) Rats pre-treated with methanol extract at doses of

125, 250 and 500 mg/kg: the lesions have reduced with the increase in doses, while at 500 mg/kg, no lesions are seen.

3.3. Indomethacin-induced ulcers (curative test)

Table 3 and figure 3 Summarize the results obtained in the experimental model of indomethacin-induced gastric ulcerations in rats. The total surface area of ulceration obtained with control was 241.70 ± 5.78 mm^2 while aqueous extract of *Distemonanthus benthamianus* stem bark provoked a significant ($p < 0.001$) dose-dependent of ulcerated area from 241.70 ± 5.78 mm^2 (distilled water n negative control group) to 144.60 ± 2.28; 35.70 ± 1.19 and 3.03±0.30 mm^2 with doses 125, 250 and 500 mg/kg, leading to an inhibition percentage of 40.18; 85.23 and 98.74% respectively. Methanol extract has also reduced ulcerated area from 112.50 ± 2.04 mm^2 (in DMSO3% negative control group) to 85.02 ± 1.01; 4.18 ± 0.40 and 0.00 ± 0.00 mm^2 at respective doses of 125, 250 and 500 mg/kg, corresponding to an inhibition percentage of 24.45; 96.30 and 100.00% respectively. Both extracts have led to similar effects as Maalox (50 mg/kg) and Misoprostol (100 µg/kg), with the respective ulcerated area of 74.28 ± 1.44 and 45.37 ± 0.84 mm^2. Mucus weights in Both extracts-treated rats have also significantly ($p < 0.001$) increased. Recorded values were equal to 37.17 ± 1.49, 54.50 ± 4.02 and 76.00 ± 3.61 mg when treated with aqueous extract and to 27.66 ± 0.13, 28.67 ± 0.62 and 84.83 ± 0.03 mg for those having received methanolic extract respectively with doses 125, 250 and 500 mg/kg, this compared with negative control groups.

Table 3: Curative effects of aqueous and methanolic extracts of *Distemonanthus benthamianus* stem bark on indomethacin-induced gastric lesions in rats.

Treatements	Dose (mg/kg)	n	US (mm^2)	%US	UI	%I	MucusWeight (mg)
Distilled water	1ml/100g b.w	6	241.70 ± 5.78	31.76 ± 1.98	5.11 ± 0.66	/	17.00 ± 1.25
DMSO 3%		6	112.50 ± 2.04	17.03 ± 0.94	3.88 ± 0.19	/	13.33 ± 2.88
Maalox	50	6	74.28 ± 1.44^{c}	10.04 ± 0.49^{c}	3.12 ± 0.20^{b}	69.26	37.33 ± 1.56^{c}
Misoprostol	100µg/kg	6	45.37 ± 0.84^{c}	6.14 ± 0.32^{c}	2.63 ± 0.19^{c}	81.23	27.50 ± 0.99
Aqueous extract of *D.benthamianus*	125	6	144.60 ± 2.28^{c}	17.37 ± 1.24^{c}	3.73 ± 0.24	40.18	37.17 ± 1.49^{c}
	250	6	35.70 ± 1.19^{c}	4.40 ± 0.38^{c}	2.77 ± 0.25^{c}	85.23	54.50 ± 4.02^{c}
	500	6	3.03 ± 0.30^{c}	0.33 ± 0.05^{c}	1.00 ± 0.00^{c}	98.74	76.00 ± 3.61^{c}
Methanol extract of *D.benthamianus*	125	6	85.02 ± 1.01^{c}	12.03 ± 0.16^{c}	3.42 ± 0.09	24.45	27.66 ± 0.13^{c}
	250	6	4.18 ± 0.40^{c}	0.62 ± 0.05^{c}	1.00 ± 0.00^{c}	96.30	28.67 ± 0.62^{c}
	500	6	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	100.00	84.83 ± 0.03^{c}

n = number of animals per group, US=Ulcerated area, %US= Ulcerate area percentages, UI= Ulcer index, %I= inhibition percentage, $^{a}p<0.05$; $^{b}p<0.01$; $^{c}p<0.001$: significant when compared to negatives controls groups (distilled water and DMSO 3%). $^{\beta}p<0.01$; $^{\gamma}p<0.001$: significant when compared to positives controls groups (Maalox, Misoprostol), DMSO =Dimethyl sulfoxide, b.w=body weight.

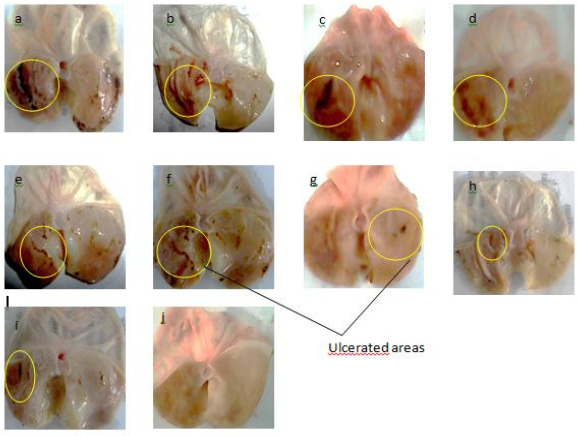


Fig. 3: Macroscopic appearance of the gastric mucosa of the rats.

(a) & (b) Distilled water and DMSO 3% pre-treated rats with 1 ml/100g (ulcer control): severe lesions are seen in the gastric mucosa; Indomethacin produced hemorrhagic necrosis of gastric mucosa. (c) & (d) Rats pre-treated with Maalox (50 mg/kg) and Misoprostol (100µg/kg) respectively: lesions in the gastric mucosa are partially comparable to those seen in negative control groups. (e), (f) & (g) Rats pre-treated with aqueous extract at doses of 125, 250 and 500 mg/kg respectively: moderate lesions are seen in the gastric mucosa, and lesions decreased when dose increased. (h), (i) & (j) Rats pre-treated with methanol extract at doses of 125, 250 and

500 mg/kg: lesions reduce with the increase of doses while at 500 mg/kg, no lesions are observed.

3.4. Acetic Acid-Induced Gastric Ulcers

Representative images of the acetic acid-induced ulcer model showed very clear ulcer margins with deep defects of the mucous layer. (Figure 4) Progressive healing of ulcers with time was defined very well in both treated groups. In this acetic acid-induced ulcer models, the initial ulcerated areas in control group having received distilled water and DMSO3% was 88.05 ± 3.29 and 74.90 ± 3.80 mm^2 respectively. Oral treatment with aqueous and methanol extracts of *Distemonanthus benthamianus* stem bark at 125 mg/kg during 14 consecutive days, have accelerated the healing of gastric ulcers with a significant ($p<0.001$) decrease of ulcerated area from 32.67 ± 0.35 and 5.78 ± 0.84 mm^2 respectively, leading to inhibition percentages of 62.90 and 92.30% respectively. The healing was complete (100% inhibition) in animals which received both extracts at doses 250 and 500 mg/kg.

Maalox and Ranitidine also significantly reduced ($p<0.001$) ulcerated area, when compared with control groups.

No significant difference ($p>0.05$) is observed in terms of the mucus weight produced between rat groups that received aqueous extract and negative control groups treated with distilled water. In contrast, mucus weight have significantly increased ($p<0.001$) in each group that received methanol extract, from 22.83 mg in negative control DMSO 3% to 84.50 ; 42.67 and 65.83 mg at the doses of 125, 250 and 500 mg/kg respectively (Table 4).

Table 4: Curative effects of aqueous and methanolic extracts of *Distemonanthus benthamianus* stem bark on acetic-acid induced gastric lesions in rats.

Treatements	Dose (mg/kg)	n	US (mm^2)	Mucus Weight (mg)	%I
Normal	/	6	0.00 ± 0.00	107.00 ± 4.27	/
Distilled water	1ml/100g b.w	6	88.05 ± 3.29	40.50 ± 4.81	/
DMSO 3%		6	74.90 ± 3.80	22.83 ± 1.11	/
Maalox	50	6	19.60 ± 0.00[c]	55.00 ± 2.25[a]	77.74
Ranitidine	50	6	18.22 ± 0.88 [c]	46.33 ± 0.80	79.31
Aqueous extract of *D.benthamianus*	125	6	32.67 ± 0.35 [c]	27.83 ± 3.09	62.90
	250	6	0.00 ± 0.00 [c]	48.33 ± 0.84	100.00
	500	6	0.00 ± 0.00 [c]	52.17 ± 1.58	100.00
Methanol extract of *D.benthamianus*	125	6	5.78 ± 0.84 [c]	84.50 ± 2.13 [c]	92.30
	250	6	0.00 ± 0.00 [c]	42.67 ± 0.95 [c]	100.00
	500	6	0.00 ± 0.00 [c]	65.83 ± 0.48 [c]	100.00

n = number of animals per group, US=Ulcerated area, %US= Ulcerate area percentages, UI= Ulcer index, %I= inhibition percentage, [a]$p<0.05$; [c]$p<0.001$: significant when compared to negatives controls groups (distilled water and DMSO 3%). DMSO =Dimethyl sulfoxyde, b.w=body weight.

3.5. Histological evaluation

The histopathology of tissues was performed and results are shown in figure 4. A section taken from the stomach of a rat treated with distilled water show the normal histological structure of mucosal layer. Stomach sections of negative control rats subjected to induction of gastric ulcer by subserosal acetic acid solution injection and respectively treated with distilled water and DMSO3%, show abnormal histological structures of mucosal layer.

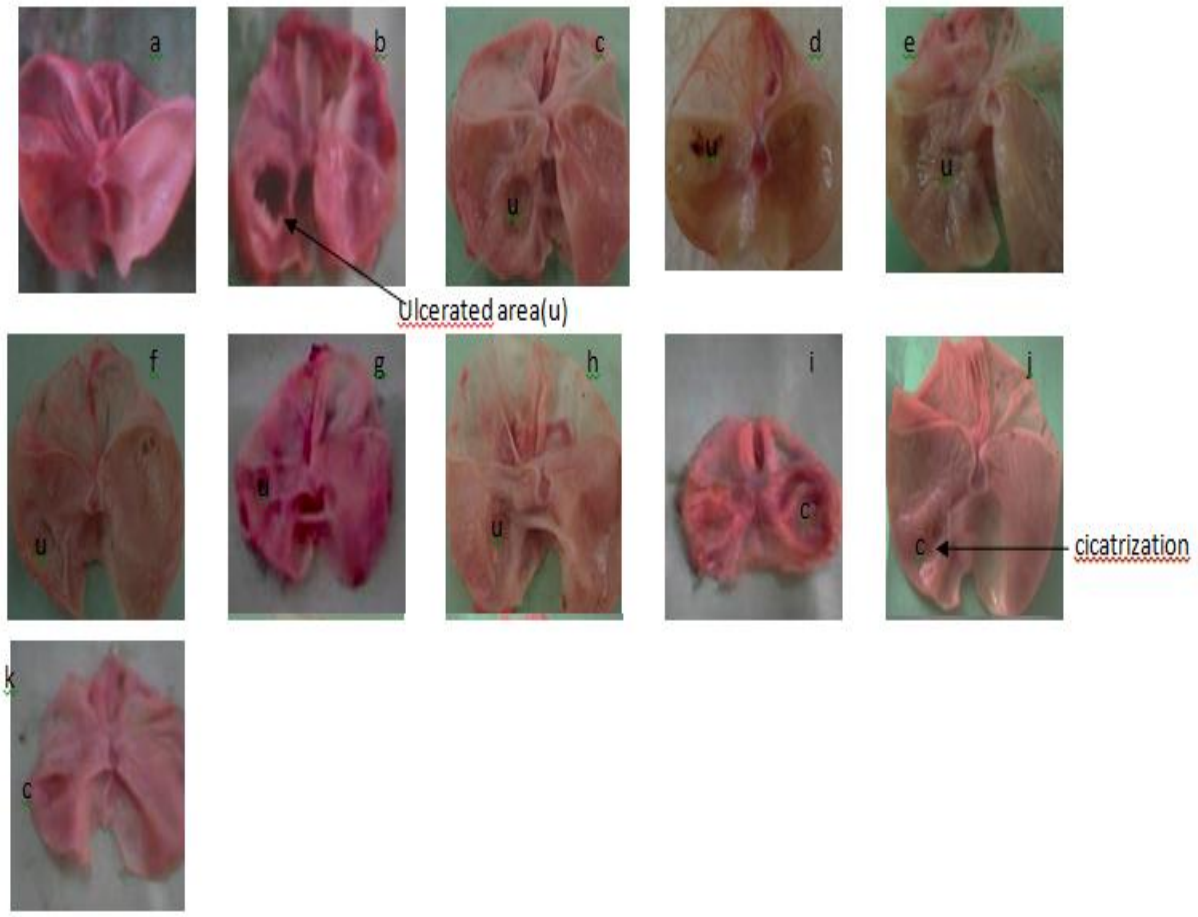
3.6. Nitric Oxide Secretion

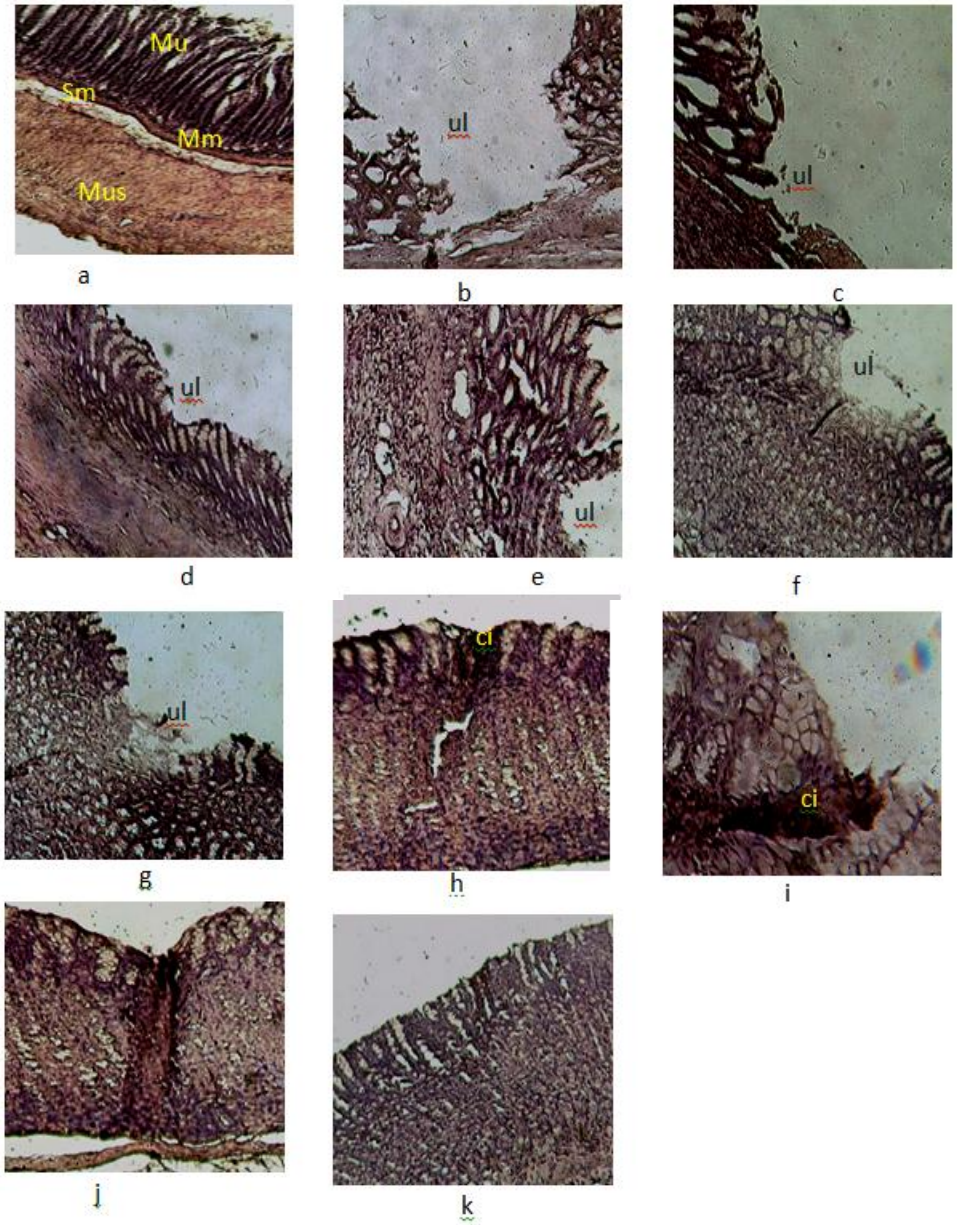
Effects of *Distemonanthus benthamianus* in nitric oxide production is represented in Table 5. No significant difference ($p>0.05$) was observed in plasma fluid when treated with aqueous extract at different doses, compared to the negative control group which received distilled water. However, the concentration of nitric oxide in the plasma fluid of rats received methanolic extract, significantly ($p<0.001$) increased from 3.95 ± 0.50 µmol/ml to 29.13 ± 10.27, 54.05 ± 3.78 and 76.38 ± 14.76 µmol/ml at the doses of 125, 250 and 500 mg/kg respectively compared to rats of the negative control group treated with DMSO 3%. Furthermore, no significant ($p>0.05$) difference was observed between the animals treated with Maalox and Ranitidine and those that received distilled water. Concerning gastric supernatant no significant difference was observed, independently of extracts used.

Table 5: Effects of aqueous and methanolic extracts of *Distemonanthus benthamianus* stem bark on nitric oxide production.

Treatment	Dose	n	[NO] Plasma (µmol/ml)	[NO] gastric supernatant (µmol/g)
Normal	/	6	8.15 ± 4.32	11.14 ± 3.61
Distilled water	1ml/100g b.w	6	3.86 ± 0.61	22.41 ± 4.00
DMSO 3%		6	3.95 ± 0.50	20.26 ± 16.38
Maalox	50	6	3.19 ± 0.40	30.85 ± 24.74
Ranitidine	50	6	5.31 ± 0.86	11.85 ± 3.12
Aqueous extract of *D.benthamianus*	125	6	4.30 ± 0.52	25.20 ± 7.73
	250	6	3.21 ± 0.41	45.68 ± 15.85
	500	6	4.05 ± 0.49	17.41 ± 5.86
Methanol extract of *D.benthamianus*	125	6	29.13 ± 10.27[c]	32.38 ± 15.36
	250	6	54.05 ± 3.78[c]	30.27 ± 9.94
	500	6	76.38 ± 14.76[c]	36.82 ± 15.46

n= number of rats per group. NO= nitric oxyde
[c]p<0.001 statistically significant relative to control animals treated with dis sulfoxyde, b.w=body weight





Mu: mucosa, ul: ulcerated area, SM: submucosa, ci: cicatrization, Mm: muscularis mucosae, Mus : muscularis externa

Fig. 4: Macroscopic observation and histological study of acetic acid-induced gastric damage in rats.

a' & a: internal structure of stomach and histological section of a normal control rat: no injuries in the gastric mucosa are seen and the gastric wall is normal. b & b' ; c & c': stomach and histological section of ulceration in control group rats: there is severe destruction of epithelial surface and necrotic lesions penetrating deeply into mucosa and sub mucosa layer. d & d': stomach and histological section of rat treated with Maalox (50 mg/kg): the gastric wall appears with necrotic layer and lack of mucosa layer in the ulcerated portion. e & e': stomach and histological section of rat treated with ranitidine (50 mg/kg): the gastric wall appears with necrotic layer and lack of epithelium layer in the ulcerated portion . f & f'; g & g': internal structure of stomach and histological section of rats treated with dose 125 mg/kg of aqueous and methanolic extracts: there is moderate disruption to the epithelium surface. h & h'; i & i': internal structure of stomach and histological section of rat treated with dose 250 mg/kg of aqueous and methanolic extracts: there is complete cicatrization of ulcerated portion. j & j'; k & k': internal structure of stomach and histological section of rat treated with dose 500 mg/kg of aqueous and methanolic extracts: there is complete cicatrization of ulcerated portion.

DISCUSSION

Ulcer has become a global disease affecting people in all geographical regions. It is generally accepted that peptic ulcer results from an imbalance between aggressive factors and the maintenance of mucosal integrity through endogenous defense mechanisms.[20] To regain the balance, different therapeutic agents including plant extracts can be used.[21] This study was conducted to evaluate the preventive and curative properties of

aqueous and methanolic extracts of *Distemonanthus benthamianus* stem bark on gastric ulcer induced by ethanol, indomethacin and acetic acid. Results obtained from this work showed that aqueous and methanolic extracts at doses 125, 250 and 500 mg/kg significantly reduced the ulceration surfaces against ulceration induced by these three models.

Lesion made by ethanol appears when its biological actions are predominant in the glandular part of the stomach. Oral administration of ethanol 95% in rats has deleterious effects on the stomach mucosa this by disabling its barrier and causing microvascular changes a few minutes after its application. A strong and rapid vasoconstriction combined with rapid arteriolar dilation induces damage in mucosal capillaries.[22] Thus, aqueous and methanolic extracts of *Distemonanthus benthamianus* stem bark have significantly ($p < 0.001$) inhibited gastric lesions in rats, meaning that those extracts could have acted in cytoprotection of the gastric mucosa. Similar results were found with aqueous and methanol extracts of *Piptadeniastrum africanum*. This could be therefore due to a reduction of acid secretion or acid neutralization.[23]

To probe the possible action mechanisms of the extracts, their antiulcer potential was tested against indomethacin-induced ulcers preventive pathway. Indomethacin is known to induce gastric ulcer by inhibition of prostaglandins which are cytoprotective for the gastric mucosa, particularly due to the inhibition of cyclooxygenase pathway of arachidonic acid metabolism, thus resulting in excessive production of leukotrienes and other products of 5-lipoxygenase pathway.[24] In the stomach, prostaglandins play a vital protective role, stimulating the secretion of bicarbonate and mucus, maintaining mucosal blood flow, and regulating mucosal cell turnover and repair. Thus, the cytoprotective effect of the anti ulcer agent when the ulcer lesions are induced by indomethacin can be mediated through endogenous prostaglandin.[25] Results obtained show that the ulcerated surface area and the mean ulcer index were significantly reduced, while the mucus weight increased in the aqueous and methanol extracts-treated groups, compared to their respective controls. Therefore, it can be thought that *Distemonanthus benthamianus* stem bark extracts may stimulate the secretion of prostaglandins or possess prostaglandins like-subtances.

In order to probe the effectiveness of *Distemonanthus benthamianus* extracts in curing gastric ulcer, their healing activities were tested against indomethacin and acetic-acid induced ulcer. It is known that indomethacin can slow down the healing process of peptic ulcer as previously said. 5 days after treatment, aqueous and methanolic extracts of bark of *Distemonanthus benthamianus* significantly increased the mucus weight as well as significantly ($p<0.001$) reduced gastric legions in rats, with percentage of cure (as it is a curative model) of 40.18; 85.23 and 98.74% for the aqueous extract, and 24.45; 96.30 and 100 % to the methanol extract at the respective doses of 125, 250 and 500 mg/kg. Therefore, aqueous and methanolic extracts stem bark of *Distemonanthus benthamianus* display an antiulcer effect related to their gastroprotective activities. Reports suggest that phytochemical compounds such as flavonoids and phenolic compounds could be active in producing antiulcer effect,[21] meaning that, the observed activities of curative ulcer of *Distemonanthus benthamianus* extracts can be due to their phytochemical constituents mainly flavonoids.

Induction of ulcer by acetic acid model was selected because this method produces stomach crater in rats similarly to that observed in human chronic ulcers. Characteristic of chronic ulcer is that damages caused by acetic acid do not only reach the mucous membrane and submucosa layers but also the muscular. Here, injuries created by acetic acid are limited, and are presented as a crater on the glandular portion of the stomach.[15] The observed significant decrease of ulcerated surface in both extracts treated animals make those extracts be active in the healing process of chronic gastric ulcer. This was proved through histological sections of the stomach of rats which received aqueous and methanolic extracts. Accelerated cell regeneration was observed in different treated groups with a complete regeneration of cells in stomach mucosa for animals which received doses 250 and 500 mg/kg of aqueous and methanolic extracts compared with both negative control groups in which crater affect the mucosa and submucosa. Curative and healing properties of those extracts could be attributed to their action on the increased production of mucus, which is an important factor in the process of ulcer healing, because the mucus protects layers against the newly formed acid and pepsin aggression.[26]

Nitric oxide (NO) is a mediator substance long considered in gastrointestinal inflammatory diseases and which play an important role in maintaining the integrity of the mucosa for the synthesis of mucus and bicarbonate.[27] Effect of aqueous and methanolic extracts on the NO secretion was evaluated in order to better appreciate the mechanisms of tissue healing of plant extracts. The determination of nitric oxide (NO) after 14 days of treatment with methanolic extract showed significant variation in plasma fluid compared to negative control group having received DMSO 3%. It is known that NO promotes ulcer healing by stimulating the formation of growth factors, regeneration of epithelial cells, angiogenesis, mucus secretion and opposing the persistence of infiltration of the mucosa by polymorphs. NO values obtained both in the plasma fluid and gastric supernatant, cancel the hypothesis that the healing process of aqueous extract of *Distemonanthus benthamianus* stems bark against chronic ulcer induced by acetic acid can be mediated through NO pathway.

CONCLUSION

The results of this study showed that the aqueous and methanolic extracts of *Distemonanthus benthamianus* stem bark possessed preventive and curative activities and could be a potential source for new antiulcer drug discovery and development. The cytoprotective action may result from the strengthening of the mucosal barrier through the increase of the mucus production. Further studies are required to confirm the exact action mechanisms underlining ulcer healing, protective properties of the extracts and identify the chemical constituents responsible for it.

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Competinginterests

There is no competing interests.

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