Trop J Nat Prod Res, September 2024; 8(9): 8546-8553 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Gastroprotective Effect of the Methanol Fraction of the Stem Bark Extract of *Entada africana* Guill. & Perr. in Wistar Rats

Millicent L. Umaru[1]*, Gabriel N. Uyaiabasi[2], Rabiu G. Tijjani[1], Celestina O. Alebiosu[3], Ekaete I. Oviawe[4], Aliyu Salihu[5], Abdullahi Sulaiman[1], Amina Y. Jega[3,6,7]

[1]*Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, P.M.B, 2346, Sokoto, Nigeria*
[2]*Department of Pharmacology, School of Basic Clinical Sciences, Babcock University, P.M.B 4003, Ilishan Remo, Ogun State, Nigeria,*
[3]*Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, P.M.B, 2346, Sokoto, Nigeria*
[4]*Department of Veterinary Surgery and Radiology, Faculty of Veterinary Medicine, Usmanu Danfodiyo University P.M.B, 2346, Sokoto, Nigeria*
[5]*Department of Histopathology, Usmanu Danfodiyo University Teaching Hospital Sokoto, P.M.B, 2346, Sokoto, Nigeria*
[6]*Center for Advanced Medical Research and Training, Usmanu Danfodiyo University, P.M.B, 2346, Sokoto, Nigeria*
[7]*One Health Institute, Usmanu Danfodiyo University, P.M.B, 2346, Sokoto, Nigeria*

ARTICLE INFO

Article history:
Received 31 July 2024
Revised 19 August 2024
Accepted 15 September 2024
Published online 01 October 2024

ABSTRACT

Peptic ulcer disease (PUD), is a debilitating condition characterized by the formation of sores in the stomach or duodenum, usually presenting with gastrointestinal pain and bleeding. *Entada africana* is used locally as herbal decoction to treat fever, dysentery and stomach ache. This study aimed to evaluate the anti-ulcer properties of the methanol stembark extract of *Entada africana* (MEA). The anti-ulcer activity of MEA was evaluated at graded doses (100, 200 and 400 mg/kg), using ethanol-induced, indomethacin-induced and pylorus-ligated (PL) ulcer models on Wistar rats. The ulcer index was calculated and histological assessments were carried out on isolated rat stomachs. MEA was shown to be safe orally. The ethanol model showed dose-dependent and statistically significant (p<0.0001) gastroprotective effects at all doses, with the highest dose of MEA (400 mg/kg) having the highest percentage inhibition of 98.1%, while omeprazole was 80.5%. Similarly, in the indomethacin model, MEA exerted a significant gastroprotective effect at the highest dose with a percentage inhibition of 74.9% while the standard drug was 85.9%. Increases in acidity and volume of acid output were observed with the PL model in all groups when compared to the control, however not statistically significant. Histological evaluation revealed regenerating mucosa at 100 and 200 mg/kg in the ethanol-induced model while the indomethacin-induced model showed almost complete re-epithelisation at the optimum dose of 200 mg/kg. Findings from this study show that MEA has good anti-ulcer and wound healing properties and this effect may be attributed to the mucosa protection rather than the anti-secretory properties.

Keywords: Peptic ulcer disease, *Entada africana*, Phytomedicine, Gastroprotective effect, Pylorus-ligation.

Introduction

Peptic ulcer disease (PUD) is a debilitating and often painful gastrointestinal disease that results from the formation of lesions or sores in the stomach/mucosal walls due to the breakage of the mucosal barrier, which exposes the underlying tissues to the corrosive actions of gastric acid and pepsin.[1-3] These open sores cause great stomach pain, upset, and internal bleeding and may sometimes escalate to gastrointestinal (GIT) perforation, obstruction of the passage of food, or cancer if left untreated or not well managed.[4,5] The aetiology and pathogenesis of PUD differ from patient to patient. PUD often occurs as a result of an imbalance between aggressive forces (primarily gastric acid and pepsin) and the defensive factors (epithelial cell regeneration, gastric blood flow, mucus secretion, prostaglandins E, bicarbonates, somatostatin, and integrity of the mucosal barrier), where the aggressive forces overwhelm the defensive factors.[3,6,7]

*Corresponding author. E mail: xonyowo@gmail.com
Tel: +2348037014269

Citation: Umaru ML, Uyaiabasi GN, Tijjani RN, Alebiosu CO, Oviawe EI, Salihu A, Sulaiman A, Jega AY. Gastroprotective Effect of the Methanol Fraction of the Stem Bark Extract of *Entada africana* Guill. & Perr. in Wistar Rats. Trop J Nat Prod Res. 2024; 8(9): 8546-8553 https://doi.org/10.26538/tjnpr/v8i9.43

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Helicobacter pylori has been shown to have a significant impact on the formation of PUD.[2,4,8] Other social habits, such as alcohol intake, smoking, eating large amounts of spicy foods, unmanaged stress, and certain medications such as non-steroidal anti-inflammatory drugs (NSAIDs), have been implicated in PUD.[9-11] The epidemiology of PUD indicates that the disease occurs worldwide with marked geographical variation; it affects at least 5 to 10% of the world's population.[12,13] The prevalence rate of PUD in sub-Saharan Africa is 24.5%; in Nigeria, it is estimated that 2.1-6.0% of the population is affected by gastric ulcers, and this rate may be on the increase.[14-16] PUD can lead to work absenteeism, high medical bills, and possible complications if not properly diagnosed and treated.[17] Treatment options for PUD include antacids (neutralise gastric acid), H_2–receptor antagonists and proton pump inhibitors (which inhibit acid secretions), mucosa protectants (such as sucralfate), or antibiotics to eradicate *H. pylori*.[2,4] The goal of treatment is to alleviate painful symptoms, promote wound healing, prevent ulcer reoccurrence, and manage complications. However, these drugs are accompanied by several adverse effects with prolonged use, drug interactions, and patient compliance, which in turn can affect their effectiveness.[4,7,18] Traditional medicinal and herbal plant supplements still play a significant role in the healthcare of the populace both in developed and developing countries.[19] In some countries, this aspect of healthcare enjoys patronage alongside commonly and widely used orthodox Medicare.[20,21] A considerable number of Nigerians rely on traditional/alternative forms of medicine for some of their healthcare needs and regularly consume these herbal concoctions.[2,22,23] However, traditional medicine has not been formally accepted or integrated into

Nigeria's healthcare system. *Entada africana* Guill. & Perr, a small tree that belongs to the family Fabaceae (Legumes), is mostly found in tropical and subtropical regions.[24] Different parts and preparations of this plant have been employed in the management and treatment of various ailments traditionally. Decoctions of the plant have been used to treat malaria, fever, dysentery, and stomach aches.[25] Infusion of the stem bark or leaves is used in wound healing, respiratory tract disorders, inflammatory liver diseases, and other diseases.[26-28] The bark of the plant is also used in making ropes and to bind storage bins. The plant is also a good source of tannin and has been shown to possess good antiseptic, astringent, haemostatic, and antiparasitic properties.[29] The wound healing, anti-inflammatory, and anti-secretory properties reported in folk medicinal use make *Entada africana* a potential remedy for PUD. This study seeks to evaluate the antiulcer activity of the methanol stem bark extract of *E. africana* (MEA) in animal models.

Materials and Methods

Drugs
Omeprazole (McCoy Pharma Pvt. Ltd., India), Indomethacin (99%, 357.79 MW, Shanghai Macklin® Biochemical Co. Ltd., Shanghai, China), Indomethacin (Geneith Pharmaceuticals, China), and Cysteamine HCL (Sigma Chemical, CO., USA). Xylazine (Xylased, Bioveta, a.s., Czech Republic), ketamine hydrochloride injection USP (Jawa International Limited, Nigeria).

Collection of plant material and preparation
The stem bark of *Entada africana* was collected from Dundaye, in Wamakko Local Government area of Sokoto State, Nigeria, in December 2022. The plant was identified and authenticated by Malam Musa Magaji at the herbarium section of the Department of Pharmacognosy and Ethnopharmacy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto, where a voucher specimen (number PCG/UDUS/Faba/0014) was deposited. The plant material was air-dried to a constant weight, pulverized, and preserved according to the method described in the African Pharmacopoeia (1985).[30] The plant material was subjected to cold maceration using 90% methanol (1 L) after defatting with hexane (500 mL). The extract was evaporated *in vacuo* using a rotary evaporator at 40°C to afford the methanol stem bark extract labelled as MEA and stored in an ambient condition (23-25°C) until required.

Experimental animals
Healthy *albino* mice (17–25 g) and *albino* Wistar rats (120–170 g) of both sexes were obtained from the animal holding unit, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria, Kaduna State, Nigeria. Animals were transported to Sokoto, where they were housed in clean and well-ventilated steel cages in the animal house facility of the Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. The animals were acclimatised for two weeks and fasted for 18–24 hours before the commencement of the study. They were maintained under standard laboratory conditions, fed with rodent pellets (diet) and clean water, room temperature of 23-25 °C, and a 12-hour light/dark cycle. The experimental protocol was approved by the Usmanu Danfodiyo University Sokoto Animal Research ethical committee, and an ethical approval number (NHREC/UDU-HREC/25/06/2023) was issued. Animals were handled according to the established public health guidelines (Guide for Care and Use of Laboratory Animals, 2011).[31]

Qualitative phytochemical screening of MEA
Preliminary phytochemical screening was carried out on MEA using the procedures described by Trease and Evans (2002)[32] and Sofowora (2008).[33] The extract was tested for the presence of tannins, alkaloids, flavonoids, carbohydrates, cardiac glycosides, phenols, anthraquinones, and triterpenoids/steroids.

Acute toxicity study
The median lethal dose (LD_{50}) of MEA was determined in *albino* mice orally using the method described by Lorke (1983).[34] In the first phase, nine mice were randomly divided into three groups consisting of three mice each. Each group was administered different doses (10, 100, and 1000 mg/kg body weights) of MEA. The animals were observed for 24 hours for both toxic effects as well as mortality. In the second phase, three (3) mice were randomly divided into three groups, and each group was administered MEA at doses of 1600, 2900, and 5000 mg/kg, respectively. The animals were again observed for another 24 hours for any signs and symptoms of toxicity and mortality. The LD_{50} was calculated as the geometric mean of the maximum dose producing no mortality and the minimum dose producing mortality.

Evaluation of antiulcer activity
Ethanol-induced gastric ulcer model
This study utilised the method described in previous studies.[3,35] Wistar rats were used for this experiment. Before the commencement of the experiment, the animals were fasted for 24 hours but had free access to water. The rats were randomly allotted to 5 groups of 5 animals each and pre-treated orally as follows:

Group 1 – Distilled water (10 mL/kg)
Group 2 – Omeprazole (20 mg/kg)
Group 3 – MEA (100 mg/kg)
Group 4 – MEA (200 mg/kg)
Group 5 – MEA (400 mg/kg)

One hour after treatment, gastric lesions were induced orally in rats with absolute ethanol (99.9%) at a dose of 8 mL/kg p.o.[3,35,36] Two hours after induction of ulcers, animals were sacrificed humanely, and their stomachs were excised and opened along the greater curvature, and washed with normal saline solution. To calculate the ulcer index, the ulcer lesions were counted using a hand lens with a transparent millimetre scale rule[37] and scored using the parameters indicated in Table 1.[5,7]

Table 1: Method of ulcer scoring/rating

Lesion	Ulcer score/rating
No lesion	0
Haemorrhage	0.5
1-3 small lesions	1
1-3 large lesions	2
3 thickened lesions	3
More than 3 small lesions	4
More than 3 large lesions	5
More than 3 thickened lesions	6

Calculation of gastroprotective/inhibition effect
The ulcer protective effect of the extract was calculated by comparing the extent of ulceration in the excised stomachs of the treated groups compared to the control group, using the average ulcer scores obtained from each treatment group with equation 1.[2,7] The isolated stomachs were preserved in formalin and histological assessment was carried out.

$$\% \text{ Gastroprotection/Inhibition} = \frac{(\text{UI in control} - \text{UI in treated})}{\text{UI in control}} \times 100 \quad \text{Eqn 1}$$

Where; UI = Ulcer index.

Indomethacin-induced gastric ulcer model
Adult Wistar rats were fasted for 36 hours and were randomly allotted into 5 groups of five (5) rats each. They were pre-treated as follows:

Group 1 – Distilled water (10 mL/kg)
Group 2 – Omeprazole (20 mg/kg)
Group 3 – MEA (100 mg/kg)
Group 4 – MEA (200 mg/kg)
Group 5 – MEA (400 mg/kg)

One hour after treatment, ulcers were induced via oral administration of indomethacin (40 mg/kg) to all the groups.[2,36] The animals were sacrificed after 6 hours and their stomachs were excised and opened as described before; the ulcer indices were calculated as highlighted above and a histological assessment was also carried out.

Pylorus ligation-induced gastric ulcers
Animals (Wistar rats) were fasted for 48 hours but had access to water, and were randomly allotted into 5 groups of 4 animals each. The animals were pre-treated orally as follows:

Group 1 – Distilled water (10 mL/kg)
Group 2 – Omeprazole (20 mg/kg)
Group 3 – MEA (100 mg/kg)
Group 4 – MEA (200 mg/kg)
Group 5 – MEA (400 mg/kg)

One hour later, the animals were anaesthetised, and a 1-inch midline abdominal incision was made below the xiphoid process. The pylorus of each animal was carefully lifted out and ligated without damaging its blood supply. The stomach was replaced and the abdominal wall sutured; the animals were allowed to recover. Eight hours after pylorus ligation (PL), the rats were sacrificed and their stomachs dissected out. The stomach content of each animal was drained into a graduated centrifuge tube and centrifuged at 1500 rpm for 30 minutes. The volume of the gastric juice supernatant was measured, pH determined, and the total acidity was determined by titrating with 0.01N NaOH using phenolphthalein as an indicator.[2,5]

Determination of the volume of acid, pH
Gastric acidity was determined by pipetting 0.1 mL of the gastric juice into a 25 mL beaker, and one drop of phenolphthalein indicator was then added and titrated with 0.01N NaOH until the appearance of a pink colour. The total volume of alkali added was noted as the volume of acid, and the pH was determined using universal indicator paper.

Statistical analysis
All results were analysed using GraphPad Prism© 9. The results are presented as mean ± standard error of the mean (SEM), and n represents the number of animals per group. Data comparison was done using one-way ANOVA followed by Dunnett's multiple comparisons test. Values were considered statistically significant at $p < 0.05$.

Results and Discussion

The phytochemical screening for MEA showed the presence of alkaloids, flavonoids, phenolic compounds and tannins, while anthraquinones were not detected (Table 2). This is consistent with findings from other studies.[29,38,39] Studies have reported that secondary metabolites such as tannins and saponins have protective effects on ethanol and indomethacin-induced gastric mucosal lesions in rats.[1,7] Polyphenol compounds have also been reported to have ulcer protective properties through various mechanisms, including increased mucus production, inhibiting HCl production, and antioxidant properties.[40] Additionally, a review paper by Falcao and colleagues reported that commonly occurring alkaloids in plants had good gastroprotective and antiulcer activities in both ethanol- and indomethacin-induced ulceration in rats and mice.[1] However, the exact mechanism of action of MEA needs to be elucidated. From the acute toxicity study, the oral median lethal dose (LD_{50}) of MEA was estimated to be greater than 5000 mg/kg, using Lorke's method, because no mortality was recorded in both phases at doses up to 5000 mg/kg, at which stage a compound may be considered to be safe. There were few signs of toxicity observed at doses of 1600, 2900, and 5000 mg/kg, respectively, such as restlessness, salivation, piloerection, bulging of the pulp, grooming, erection of the pinna, heavy breathing, and pale colouration of the eye. However, no mortality was observed in any of these groups.

The gastroprotective effect of MEA and the standard drug in the ethanol-induced gastric ulcer model is indicated in Figure 1. MEA exhibited a dose-dependent antiulcer effect, with the highest protection obtained at 400 mg/kg, which exhibited 98.1% inhibition compared to 80.5% for omeprazole. All MEA treatment groups performed better than omeprazole (Table 3). This result showed that MEA significantly ($p < 0.0001$) reduced ethanol-induced ulcerations at all doses. Ethanol is known to induce gastric lesions through its corrosive effects, which disrupt the mucous-bicarbonate protective barrier and expose the underlying mucosa to damage from hydrochloric acid and pepsin. It also produces a massive intracellular accumulation of calcium, which represents a major step in the pathogenesis of gastric mucosal injury.

Table 2: Phytochemical constituents of MEA

S/N	Constituents	Test	Inference
1.	Carbohydrates	Molisch's	+
		Fehling's	+
2.	Alkaloids	Mayer's	+
		Dragendroff's	+
		Hager's	+
3.	Saponins	Frothing	+
4.	Cardiac glycosides	Killer-Killiani's	+
5.	Flavonoids	Ferric chloride	+
		Alkaline	+
		Shinoda	+
6.	Anthraquinones	Bontrager's	-
		Modified Bontrager	-
7.	Tannins	Ferric chloride	+
		Lead acetate	+
8.	Triterpenoid/steroids	Salkowki's	+
		Liberman-Burchad's	+
9.	Phenolic compound	Ferric chloride	+

Key: + (present); - = not detected; MEA= Methanol stem bark extract of *Entada africana*.

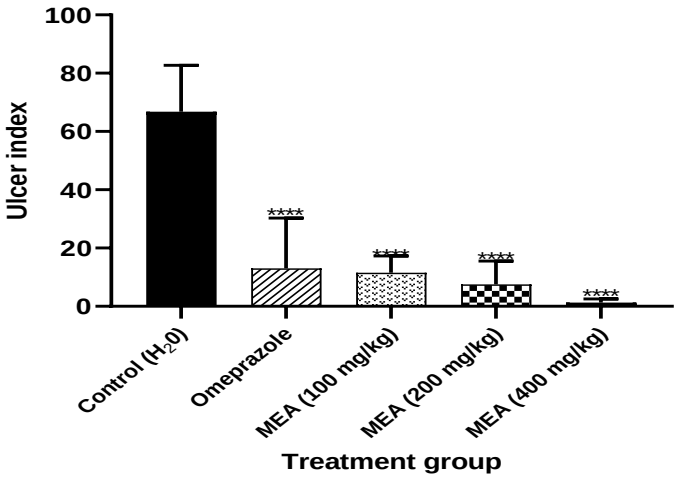

Figure 1: Bar graph showing ulcer indices of ethanol-induced ulceration in rats
Key: MEA = methanolic fraction of the stem bark of *E. africana.* Values are mean ± SEM (n = 4). Highly statistically significant reductions ****$p < 0.0001$, for both MEA and omeprazole when compared to control (One-way ANOVA followed by Dunnett's multiple comparison tests).

Table 3: Gastroprotective effects of MEA and omeprazole against ethanol – and indomethacin-induced gastric ulcers in Wistar rats

		Ethanol		Indomethacin	
Treatment	**Dose (mg/kg)**	**Ulcer index**	**% inhibition**	**Ulcer index**	**% inhibition**
Control (H_20)	10 (mL/kg)	66.75	-	33.8	-
Omeprazole	20	13	80.5	4.8	85.9
MEA	100	11.5	82.8	22.5	33.4
MEA	200	7.5	88.8	17.8	47.5
MEA	400	1.25	98.1	8.5	74.9

Key: MEA = methanolic fraction of the stem bark of *E. africana*, % inhibition for the treated groups was calculated with respect to the control.

This leads to cell death and exfoliation in the surface epithelium.[3-5,8] The high level of gastroprotection demonstrated by MEA may be attributed to its efficacy in maintaining the mucus-bicarbonate barrier of the GIT, thereby shielding the mucosa from the damage by hydrolytic and photolytic effects of gastric juices. Similarly, in the indomethacin model, MEA exerted a significant (p = 0.006) cytoprotective effect at the highest dose of 400 mg/kg with percentage inhibition of 74.9%, while the standard drug omeprazole was statistically significant (p<0.0002) with percentage inhibition of 85.9% when compared to the control (Table 3). However, the lowest dose of MEA (100 mg/kg) did not show statistically significant (p = 0.1285) protection in this model (Figure 2). The indomethacin-induced ulcer model is widely used for investigating the anti-secretory and cytoprotective activities of target agents or compounds.[2,16,40] Indomethacin is reported to induce stomach ulcerations by causing inflammation of the gastric mucosa and accumulation of reactive oxygen species, which causes damage to the mucosa.[10,41] This is in addition to the commonly known mechanism of inhibition of prostaglandin production by NSAIDs. Indiscriminate use of NSAIDs has been implicated as the second most common cause of gastric ulcers after *Helicobacter pylori*.[11-12,15,41] The cytoprotection offered by MEA was comparable to the standard drug omeprazole. Furthermore, the effect of pylorus-ligation causes the accumulation of acid in the stomach and models the effect of stress, which is a precipitating factor for gastric ulcers.[3,7] The effect of MEA on gastric pH and acid volume showed an increase in both acidity and volume of acid output when compared to the control. This was also observed for the standard drug group. These increases were, however, not statistically significant when compared to the control, with a significant effect recorded only for total acid output for MEA at 400 mg/kg (p = 0.0052) treatment group (Table 4).

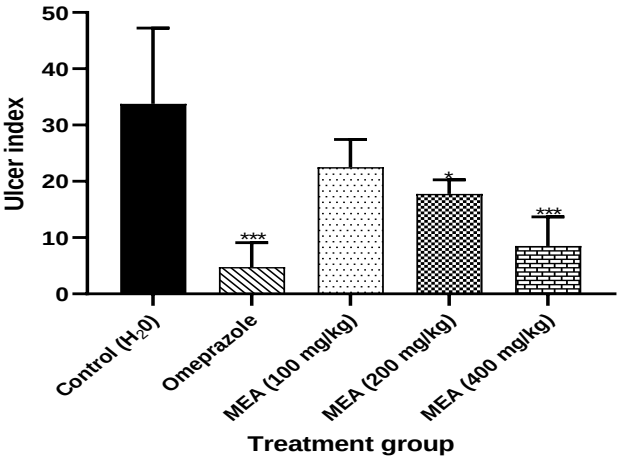

Figure 2: Bar graph showing ulcer indices of indomethacin-induced ulceration in rats
Key: MEA = methanolic fraction of the stem bark of *E. africana.* Values are mean ± SEM (n = 4). Statistically significant reductions *** p = 0.0002 for omeprazole; * p = 0.0220, p = 0.006 for 200 & 400 mg/kg respectively when compared to control. (One-way ANOVA followed by Dunnett's multiple comparison tests).

Table 4: Gastric pH values and volumes of the treated group following pylorus-ligation

Treatment	Dose (mg/kg)	Gastric volume (ml/4 h)	Gastric pH	Titratable acidity (mEq/L)	Total acid output (mEq/L)
Control (H_20)	10 (mL/kg)	0.35 ± 0.17	6.13 ± 0.24	23.7 ± 10.32	69 ± 7.72
Omeprazole	20	0.53 ± 0.08	4.88 ± 1.13	53.5 ± 15.96	96 ± 15.23
MEA	100	0.30 ± 0.06	5.00 ± 0.58	35.4 ± 7.786	117 ± 13.99
MEA	200	0.38 ± 0.09	3.75 ± 0.48	40.8 ± 12.42	101 ± 12.58
MEA	400	0.38 ± 0.11	4.50 ± 0.29	50.9 ± 13.1	143 ±16.36**

Key: MEA = methanolic fraction of the stem bark of *E africana.* Values are mean ± SEM (n = 4). Statistically significant reductions ** p = 0.0052 (One-way ANOVA followed by Dunnett's multiple comparison tests).

This pattern of increase in acid volume, when compared with the control, has been reported in other studies,[7,15] while others reported a reduction in acidity and acid volume. [3,5,8] The histological findings from the isolated stomachs of rats indicated significant mucosal regeneration and ulcer healing both in the ethanol- and indomethacin-induced gastric ulceration after treatment with MEA and the standard drug (omeprazole). For the ethanol model, only the control group showed mucosa ulceration; all the treated groups were protected (Table 5, Plate 1). In a similar vein, the histological findings for ethanol-induced ulceration showed that MEA has a gastroprotective effect at lower concentrations, but there was severe ulcerative gastritis at a dose of 500 mg/kg (Table 5 and Plate 1 a-e). For the indomethacin-induced ulceration, histological findings showed that MEA had good gastroprotective effects, with the maximum effect seen at 200 mg/kg dose, which showed significant wound healing and almost complete reepithelization. Details of the effects are shown in Table 6 and Plate 2 (a-e).

Table 5: Histological assessment of rat stomachs in ethanol-induced gastric ulcer model

Investigation	Control Distil H_2O (10 ml/kg)	Omeprazole (20 mg/kg)	MEA (100 mg/kg)	MEA (200 mg/kg)	MEA (400 mg/kg)
Overall architecture	Maintained	Maintained	Maintained	Maintained	Maintained
Mucosal Ulceration (+/-)	+	-	-	-	+
Mucosal Erosion (+/-)	-	+	+	++	-
Mucosal \oedema (+/-)	+	+	+	+	+
Submucosal oedema (+/-)	+	+	+	+	+
Neutrophilic Infiltrates	Moderate	Moderate	Moderate	Moderate	Severe
Lymphoplasma cells Infiltrates	Mild	Mild	Mild	Mild	+
Fibrosis (+/-)	Moderate	Mild	Mild	Mild	Mild
Diagnosis	Ulcerative Gastritis	Mild Erosive Gastritis	Mild Erosive Gastritis	Moderate Erosive Gastritis	Severe Ulcerative Gastritis

Key: MEA = methanolic extract of stem bark of *E. africana*, + = present), - = not present

Table 6: Histological assessment of rat stomachs in indomethacin-induced gastric ulcers model

Investigation	Control Distil H_2O (10 ml/kg)	Omeprazole (20 mg/kg)	MEA (100 mg/kg)	MEA (200 mg/kg)	MEA (400 mg/kg)
Overall architecture	Maintained	Maintained	Maintained	Maintained	Maintained
Mucosal Ulceration (+/-)	+	-	-	-	-
Mucosal Erosion (+/-)	-	+	+	-	+
Mucosal oedema (+/-)	+	+	+	-	-
Submucosal oedema (+/-)	+	+	+	-	+
Neutrophilic Infiltrates	Moderate	Mild	Mild	Mild	Mild
Lymphoplasma cells Infiltrates	Moderate	Mild	Mild	Mild	Mild
Fibrosis (+/-)	Mild	-	-	-	-
Diagnosis	Ulcerative Gastritis	Mild Erosive Gastritis	Mild Erosive Gastritis	Mild Superficial Gastritis	Mild Erosive Gastritis

Key: MEA = methanolic extract of stem bark of *Entada africana*, + = present), - = not present

The anti-ulcer effect of MEA can be said to be through mucosal epithelial regeneration and ulcer healing. This suggests that MEA possesses more cytoprotective and wound-healing properties than anti-secretory properties. Further isolation of the components of MEA may yield potential lead compounds that could be developed into standard anti-ulcer drugs. These potential new compounds can be used alone or in combination with other currently used anti-secretory ulcer drugs such as omeprazole, cimetidine, or ranitidine due to their good cytoprotective and healing properties. Non-communicable diseases such as cardiovascular, respiratory, obesity, and peptic ulcer diseases are increasing globally.[17,42] Some factors contributing to this observed increase include lifestyle habits, such as adopting more sedentary routines, unhealthy eating habits, stress, smoking, large intakes of alcohol, and climate change.[43-45] In developing countries such as Nigeria, additional factors of poverty and poor nutrition may aggravate these diseases.[42]

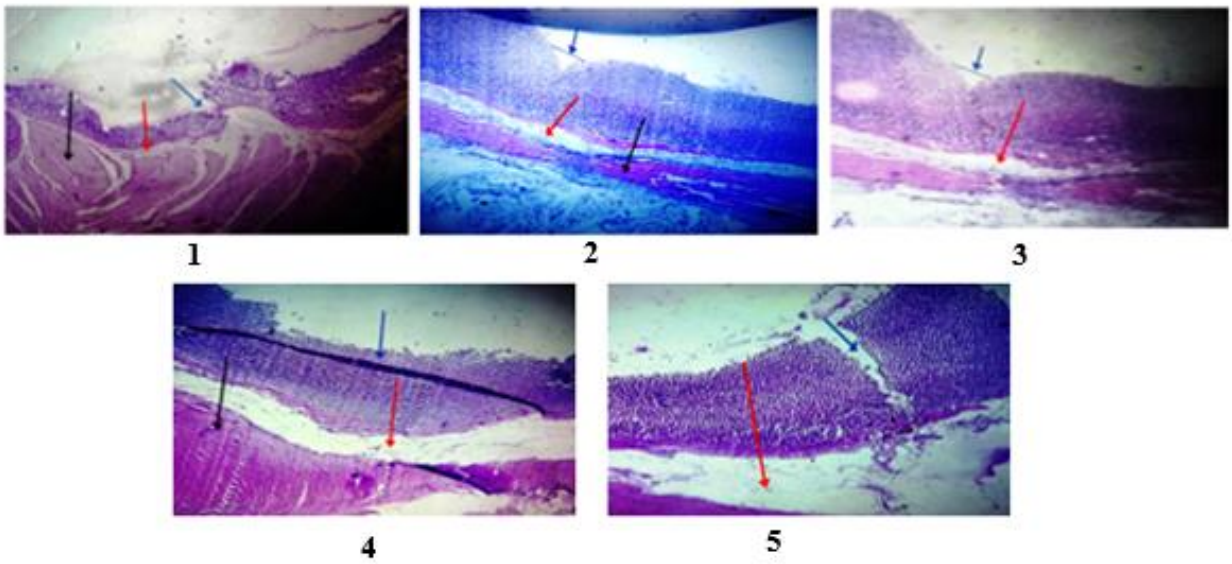

Plate 1: Hematoxylin and eosin photomicrographs of groups 1 to 5 showing representative sections of the stomach of ethanol-induced ulcerations (H&E x100 magnification). Group 1 (Distilled Water) pictograph showing ulcerations in mucosal, underlying submucosa and muscularis, Group 2 (Omeprazole 20 mg/kg) pictograph shows regenerating mucosa, submucosa and muscularis, Group 3 (MEA 100 mg/kg) showing regenerating mucosa, submucosa and muscularis, Group 4 (MEA 200 mg/kg) showing regenerating mucosa, submucosa and muscularis, Group 5 (MEA 400 mg/kg) showing ulcer edge and oedematous submucosa.
KEY: MEA - methanolic extract of stem bark of *E. africana*, blue arrow – mucosa, red arrow – submucosa, black arrow – muscularis

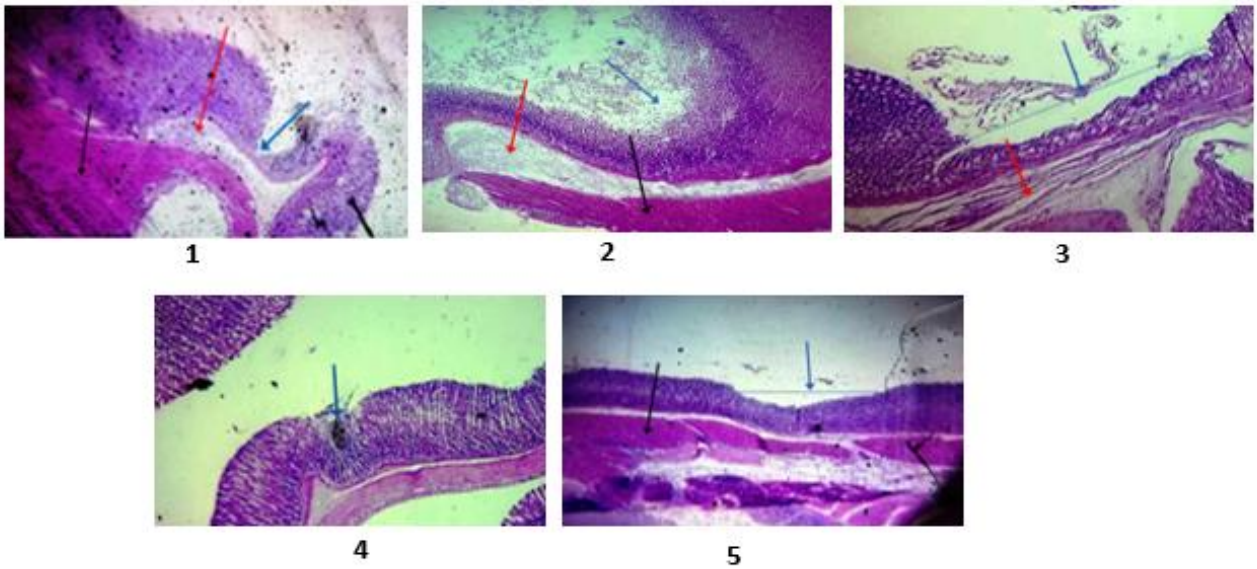

Plate 2: Hematoxylin and eosin photomicrographs of groups 1 to 5 showing representative sections of the stomach of indomethacin-induced ulcerations (H&E x100 magnification). Group 1 (Distilled Water) pictograph showing gastric ulcer in the mucosa, underlying oedematous submucosa and muscularis, Group 2 (Omeprazole 20 mg/kg) showing regenerating mucosa, submucosa and muscularis, Group 3 (MEA 100 mg/kg) mucosal epithelial regeneration and submucosa Group 4 (MEA 200 mg/kg) showing ulcer healing with complete re-epithelisation, Group 5 (MEA 400 mg/kg) showing regenerating mucosa and muscularis.
KEY: MEA - methanolic extract of stem bark of *E. africana*, blue arrow – mucosa, red arrow – submucosa, black arrow – muscularis

Conclusion

The gastroprotective activity of MEA was investigated using three *in vivo* ulcer models. MEA showed significant dose-dependent gastroprotective and healing activities compared to the standard drug (omeprazole) in ethanol-induced ulcer and indomethacin-induced models with high percentage inhibition. MEA demonstrated a good safety profile and contained important secondary metabolites that have been reported to have good gastroprotective and other medicinal properties. Further studies are necessary to establish the viability of some constituents of MEA as potential anti-ulcer lead targets.

Conflict of Interest

The authors declare no conflict of interest.

Author's Declaration

The author hereby declares that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by him.

Acknowledgements

The authors wish to appreciate Mal. Abubakar Musa Garba of the College of Health Technology, Minna, Mal. Abubakar Muhammad and Mrs. Chioma Ndubuizu of the Department of Pharmacology and Toxicology, Mal. Aliyu A. Garba of the Department of Pharmacognosy and Ethnopharmacy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto – Nigeria for their technical assistance.

References

1. Falcao HD, Leite JA, Filho JMB, Athayde-Filho PF, Chaves MCA, Moura MD, Ferreira AL, Almeida ABA, Souza-Brito ARM, Diniz MDFM, Batista LM. Gastric and Duodenal Antiulcer Activity of Alkaloids: A Review. Mol. 2008; 13(12):3198-3223.
2. Adinortey MB, Ansah C, Galyuon I, Nyarko A. *In vivo* models used for evaluation of potential antigastroduodenal ulcer agents. Ulcers. 2013, 12:796405, doi: 10.1155/2013/796405.
3. Agbaje EO, Fageyinbo MS, Alabi OO. Gastro-duodenal protective effect of aqueous leaf extract of *Daucuscarota sativus* Linn. (Apiaceae) in rats and its possible mechanism of action. J Phytopharmacol. 2017; 6(3):156-163.
4. Paragomi P, Dabo B, Pelucchi C, Bonzi R, Bako AT, Sanusi NM, Nguyen QH, Zhang ZF, Palli D, Ferraroni M, Vu KT, Yu GP, Turati F, Zaridze D, Maximovitch D, Hu J, Mu L, Boccia S, Pastorino R, Tsugane S, Hidaka A, Kurtz RC, Lagiou A, Lagiou P, Camargo MC, Curado MP, Lunet N, Vioque J, Boffetta P, Negri E, La Vecchia C, Luu HN. The Association between Peptic Ulcer Disease and Gastric Cancer: Results from the Stomach Cancer Pooling (StoP) Project Consortium. Cancers (Basel). 2022; 14(19):4905. doi: 10.3390/cancers14194905.
5. Agbaje E, Doe P. Gastric and duodenal antiulcer effects of aqueous bark extract of *Dialium guineense* Wild. (Fabaceae) and the possible mechanisms in laboratory models. J Phytopharmacol. 2015; 4(5):268-275. doi: 10.31254/phyto.2015.4506.
6. Sharifi-Rad M, Fokou P, Sharopov F, Martorell M, Ademiluyi A, Rajkovic J, Salehi B, Martins N, Iriti M, Sharifi-Rad J. Antiulcer agents: from plant extracts to phytochemicals in healing promotion. Mol. 2018; 23(7):1751.
7. Ugwah MO, Ugwah-Oguejiofor CJ, Etuk EU, Bello SO, Aliero AA. Evaluation of the antiulcer activity of the aqueous stem bark extract of *Balanites aegyptiaca L* Delile in Wistar rats. J Ethnopharmacol. 2019; 239:111931. doi: 10.1016/j.jep.2019.111931.
8. Okpo SO, Orhue JNE, Falodun A, Alonge PO, Airemwen CO. Evaluation of the gastroprotective activity of 3-carbomethoxypyridine from *Pyrenacantha staudtii Engl*. (Icacinaceae) in rats. J Pharm Bioresourc. 2012; 9(2):85-91. doi: 10.4314/jpb.v9i2.5
9. Agofure O, Okandeji-Barry O, Odjimogho S. Prevalence of Alcohol Consumption among Primary and Secondary School Adolescents in Obiaruku Community in Delta State, Southern Nigeria. Afr J Biomed Res. 2020; 23:35-40.
10. Huang JQ, Sridhar S, Hunt RH. Role of *Helicobacter pylori* infection and non-steroidal anti-inflammatory drugs in peptic ulcer disease: a meta-analysis. *Lancet.* 2002; 359(9300):14-22.
11. Narayanan M, Reddy KM, Marsicano E. Peptic Ulcer Disease and *Helicobacter pylori* infection. Mol Med. 2018; 115(3):219-224.
12. Lanas A and Chan FKL. Peptic ulcer disease. Lancet. 2017; 390(10094):613-624.
13. Snowden FM. Emerging and reemerging diseases: a historical perspective. Immunol Rev. 2008; 225(1):9-26.
14. Nwokediuko CS, Ijoma U, Obienu O, Picardo N. Time trends of upper gastrointestinal diseases in Nigeria. Ann Gastroenterol. 2012; 25(1):1-5.
15. Okpo SO, Akinrinde AO, Ottih AE, Anazodo U and Chijioke MC. Evaluation of gastroprotective efficacy and safety profile of low dose cimetidine and L-glutamine using gastric and duodenal ulcer models in rats. Afr J Pharm Res Develop 2016; 8(1):44-55.
16. Ray-Offor E and Opusunju KA. Current status of peptic ulcer disease in Port Harcourt metropolis, Nigeria. Afr Health Sci. 2020; 20(3):1446-1451.
17. Abbasi-Kangevari M, Ahmadi N, Fattahi N, Rezaei N, Malekpour MR, Ghamari SH, Moghaddam SS, Azadnajafabad S, Esfahani Z, Kolahi AA, Roshani S, Rezazadeh-Khadem S, Gorgani F, Naleini SN, Naderimagham S, Larijani B, Farzadfar F. Quality of care of peptic ulcer disease worldwide: A systematic analysis for the global burden of disease study 1990-2019. PLoS ONE; 2022; 17(8):e0271284. doi: 10.1371/journal.pone.0271284
18. Ugwah-Oguejiofor CJ, Inuwa FS, Abubakar K, Ugwah OM, Mshelia EH, Umar M. (). Evaluation of antiulcer activity of the aerial parts of *Caralluma dalzielii* N. E. Brown in Wistar rats. Trop J Nat Prod Res. 2017 1(4):168–172. doi.org/10.26538/tjnpr/v1i4.5.
19. Agbedahunsi JM. Screening of Crude Drugs for the Treatment of Malaria in Nigeria, Phytomedicine in Malaria and Sexually Transmitted Diseases: Challenges for the new millennium. Drug Research and Production Unit, Faculty of Pharmacy, Obafemi Awolowo University, Ile Ife, Nigeria; 2000. 13 – 22 p.
20. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol. 2014; 4:177.
21. Hu R, Lin C, Xu W, Liu Y, Long C. Ethnobotanical study on medicinal plants used by Mulam people in Guangxi, China. J Ethnobiol Ethnomed. 2020; 16(1):1-50.

22. Odugbemi TO, Akinsulire OR, Aibinu IE, Fabeku PO. Medicinal plants useful for malaria therapy in Okeigbo, Ondo State, Southwest Nigeria. Afr J Tradit Complement Altern Med. (2006) 13; 4(2):191-198. doi: 10.4314/ajtcam.v4i2.31207.
23. Adebisi IM, Bakare UA, Umaru ML. Medicinal Plants Used in the Treatment of Pregnancy Related Problems in Sokoto - State, Nigeria. Trop J Nat Prod Res. 2018; 2(11):466-470. doi.org/10.26538/tjnpr/v2i11.1
24. Baidoo MF, Evelyn AK, Abraham YM, George HS, Amponsah IK. Pharmacognostic characterization and development of standardization parameters for the quality control of *Entada africana* Guill. & Perr. J Appl Res Med. 2019; 12:36-42. doi: 10.1016/j.jarmap.2018.11.003.
25. Orwa C, Mutua A, Kindt R, Jamnadass R, Anthony S. Agroforestry Database: A Tree Reference Selection Guide Version 4.0 [online]. 2009 [cited 2024 Feb.21] Available from: https//www.worldagroforestr.org/treedbs/treedatabases.asp.
26. Occhiuto F, Sanogo R, Germano P, Keita A, D'Angelo V, De Pasquale R. Effects of some Malian medicinal plants on the respiratory tract of guineapigs. J Pharm Pharmacol. 1999; 51:1299-1303.
27. Mvondo MA, Essono SM, Tatsinkou FDB, Ateba SB, Dieudonné N, The Root Aqueous Extract of *Entada africana* Guill. Et Perr. (Mimosaceae) Inhibits Implant Growth, Alleviates Dysmenorrhea, and Restores Ovarian Dynamic in a Rat Model of Endometriosis. Evid-Based Complement Alternat Med. 2017; 2017:8563909, doi.org/10.1155/2017/8563909.
28. Kwaji A, Adamu H, Chindo I. Phytochemical analysis, antibacterial and antioxidant activities of *Entada africana* Guill. and Perr. Stem bark extracts. J Chem Sci. 2017; 7(10):10–15.
29. Yusuf AJ and Abdullahi MI. The phytochemical and pharmacological actions of *Entada africana Guill. & Per*r. Heliyon. 2019; 5(9):e02332. doi: 10.1016/j.heliyon.
30. African Pharmacopoeia; Organization of African Unity, Scientific, Technical & Research Commission: Lagos, Nigeria, 1985; Volume 1.
31. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals. 8th ed. Washington (DC): National Academies Press (US); 2011.
32. Trease GE and Evans WC. Phytochemicals in Pharmacognosy, (15th ed.). London: Saunders Publishers; 2002. 42-44, 221-229, 246-249, 304-306, 331-332, 391-393 p.
33. Sofowora A. Medicinal Plants and Traditional Medicine in Africa (3rd ed.). Ibadan: Spectrum Books Limited; 2008. 162-179 p.
34. Lorke D. A new approach to practical acute toxicity testing. Arch. Toxicol. 1983; 54:275-287.
35. Guzmán-Gómez O, García-Rodríguez RV, Quevedo-Corona L, Pérez-Pastén-Borja R, Rivero-Ramírez NL, Ríos-Castro E, Pérez-Gutiérrez S, Pérez-Ramos J, Chamorro-Cevallos GA. Amelioration of Ethanol-Induced Gastric Ulcers in Rats Pretreated with *Phycobiliproteins of Arthrospira* (Spirulina) Maxima. Nutrients. 2018; 10(6):763. doi: 10.3390/nu10060763.
36. Meena DK and Jayanthi M. *In-Vivo* Models Used for Pre-Clinical Evaluation of Anti-Ulcer Activity. Austin Pharmacol Pharm. 2018; 3(2):1017.
37. Magaji RA, Okasha MAM, Abubakar MS, Fatihu MY. Anti-ulcerogenic and antisecretory of the n-butanol portion of *Syzygium aromaticum* in rats. Nig J Pharm Sci. 2007; 6(2):119-126.
38. Codo-Toafode M, Ahyi V, Marquardt P, Fester K. Characterization of extracts of *Entada africana* – a plant traditionally used for the treatment of musculoskeletal disorders in Benin. Z. Phytother. 2017; 38(1):S1–S44.
39. Hassan LG, Mshelia HE, Umar KJ, Kangiwa SM, Ogbiko C, Yusuf AJ. Analgesic activity and toxicity profile of the ethylacetate extract of the stem bark of *Entada africana* (Fabaceae) Guill. et Perr. ASUU J Sci.-A J Res Dev. 2017; 4(1):114–121.
40. Madueke AC, Ugwu DO, Nwanelo VO, Anosike AC, Anajemba RC, Onoh PA. Anti-Ulcerogenic Effect of Unripe Plantain (*Musa Paradisiaca*) Pulp on Indomethacin-Induced Ulcer in Wistar Rat. Trop J Nat Prod Res. 2020; 4(10):784-789. doi: 10.26538/tjnpr/v4i10.20.
41. Lawal F, Garba K, Shuai'bu AB, Chedi BAZ. Evaluation of The Antiulcer Activity of Aqueous Seed Extract of *Moringa Oleifera Lamarck* (Moringaceae). Trop J Nat Prod Res. 2018; 2(3):140–144. doi.org/10.26538/tjnpr/v2i3.8.
42. World Health Organization. Global Health Estimates: Life expectancy and leading causes of death and disability for 2019. [Online]. 2020 [cited 2024 July 12] Available from https://www.who.int/data/gho/data/themes/mortality-and-global-health-estimates.
43. Rahman Z, Dwivedi D, Jena G. Ethanol-induced gastric ulcer in rats and intervention of tert-butylhydroquinone: Involvement of Nrf2/HO-1 signalling pathway. Hum Exp Toxicol. 2020; 39(4):547-562. doi:10.1177/0960327119895559.
44. Adedeji WA, Dairo MD, Nguku PM, Oyemakinde A, Fehintola FA. Pattern and predictors of medication use among adults in southwestern Nigeria: A community-based cross-sectional study. Pharmacol Res Perspect. 2023; 11(1):e01017. doi: 10.1002/prp2.1017.
45. Lanas Á, Carrera-Lasfuentes P, Arguedas Y, García S, Bujanda L, Calvet X, Ponce J, Perez-Aísa Á, Castro M, Muñoz M, Sostres C, García-Rodríguez LA. Risk of upper and lower gastrointestinal bleeding in patients taking nonsteroidal anti-inflammatory drugs, antiplatelet agents, or anticoagulants. Clin Gastroenterol Hepatol. 2015; 13(5):906-912. e2.