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Research Article

Gastroprotective Effects of the Aqueous Seed Extract of *Entada gigas* (Linn.) Fawc. and Rendle (Fabaceae) in Ulcer Models in Rats

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Background: The extract of *Entada gigas* Linn. (Fabaceae) is used for the treatment of ulcer in Nigerian local medicine.

Objectives: This study investigated the gastroprotective effects of the aqueous seed extract of *E. gigas* on gastric ulcers in rats.

Methodology: The ethanol- (EIU), pylorus ligation- (PLIU) and cold restraint stress (CRSIU)-induced ulcer models were used. The aqueous seed extract was administered at doses of 50-400 mg/kg *p.o*. Estimations of gastric content volume, pH and titratable acidity in PLIU test and ulcer score/index in all models were done.

Results: In the EIU test, *E. gigas* produced significant reduction ($p < 0.05$) in ulcer scores, with peak effect elicited at 100 mg/kg (2.30 ± 0.99; 87.22% inhibition), compared with control (10.80 ± 0.80). This effect was comparable to that of misoprostol (3.00 ± 1.31; 83.33% inhibition). In the PLIU test, the extract caused significant reduction ($p < 0.05$) in the ulcer score compared with control (6.30 ± 0.70). Peak effect was elicited at the dose of 200 mg/kg (ulcer score 2.90 ± 0.83; 63.17%). This effect was comparable to that of cimetidine (2.60 ± 0.93; 66.98%). Also, the extract at 50 and 200 mg/kg, and cimetidine 100 mg/kg (0.24 ± 0.07, 1.06 ± 0.22 and 1.02 ± 0.16 mL/4 h, respectively) significantly reduced ($p < 0.05$) the volume of gastric content relative to control (2.32 ± 0.33 mL/4 h). In the CRSIU test, *E. gigas* caused significant reduction ($p < 0.05$) in the ulcer score, with the greatest effect produced at the dose of 50 mg/kg (0.80 ± 0.49; 92.38% inhibition), relative to control (4.20 ± 0.64). This effect was comparable to that of misoprostol (1.40 ± 0.60; 80% inhibition).

Conclusion: The aqueous seed extract of *E. gigas* possess significant antiulcer activity mediated via cytoprotective and anti-secretory mechanisms.

Keywords: *Entada gigas*, Fabaceae, ulcer, gastroprotective effect, cytoprotective, anti-secretory.

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1. Introduction

Peptic ulcer is a common gastrointestinal disorder with high morbidity rate (Falk, 2001) and it results from established imbalance between the aggressive factors (e.g. gastric acid, pepsin and *Helicobacter pylori*) and defensive factors (e.g. bicarbonate secretion, prostaglandins, gastric mucus and innate resistance of the mucosal cells) (Dashputre and Naikwade, 2011; Srinivas et al, 2013). The causes of peptic ulcer include

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infection with *H. pylori*, stress, injury/necrosis of mucus neck cells, excess acid production in the stomach and chronic use of aspirin and non-steroidal anti-inflammatory drugs (NSAIDs) (Zdanowicz, 2002).

Approximately 70% of the world population currently uses medicinal herbs as complementary or alternative medicine (Wills et al 2000; Fasinu et al, 2012). In recent years, focus on plant research has increased worldwide and several studies had showed immense potential of medicinal plants (Dahanukar et al, 2000). Herbal medicines are increasingly being recognized in treating various diseases, with relatively little knowledge of their modes of action (Begum et al, 2008), and the use of natural drugs in gastric ulcer has been reported (Sairam et al, 2001). Antiulcer chemical principles that have been isolated from plants include glycyrrhetinic acid from *Glycyrrhiza glabra* (Srinivas et al, 2013), populnoic acid from *Austroplenckia populnea* (Andrade et al, 2006), and the sesquiterpene lactone- tagitinin C from *Tithonia diversifolia* (Sánchez-Mendoza et al, 2011).

Orthodox drugs available for the treatment of peptic ulcer disease such as anticholinergics, histamine H_2-receptor antagonists, antacids and proton-pump inhibitors cause many adverse effects (Adinortey et al, 2013). Proton pump inhibitors (e.g. omeprazole and lansoprazole) may cause nausea, abdominal pain, constipation, diarrhea and H_2-receptor antagonists (cimetidine) may cause gynecomastia and loss of libido (Srinivas et al, 2013). This has led to the search for herbal medicines possessing fewer side effects as alternatives for the treatment of peptic ulcer disease. This justifies the search for herbal remedies and new drugs with better or comparable efficacy and improved safety profile.

Entada gigas (Linn.) Fawc. and Rendle (Fabaceae) is a liane with stout long stem climbing to the canopy of the evergreen and deciduous forests of West Africa. The plant is commonly called "Sea heart", "Monkey ladder vine", "Coco-de-Mer", and "Escalera de mono", with the local name being "Aagba" (Yoruba; south-west Nigeria). *E. gigas* is commonly found in West Africa from Guinea-Bissau to Southern Nigeria and generally widespread in tropical Africa, Asia and N Australia. In Nigeria, a preparation of the aqueous seed extract of *E. gigas* is used in the treatment of gastrointestinal disorders, especially diarrhea and ulcer (Gunn and Dennis, 1976). The antimicrobial activity of *E. gigas* has been reported (Fankam et al, 2014). This study was designed to evaluate the gastroprotective effects of the aqueous seed extract of *E. gigas*, based on use in traditional medicine in Nigeria, on gastric ulcers induced in rats.

2. Methodology

2.1 Drugs and Chemicals

Cimetidine (Yanzhou Xier Kangtai Pharmaceutical Co. Ltd., China), misoprostol (Cytotec®, Pfizer Pharmaceuticals, NY, USA), indomethacin (Yangzhou No. 3 Pharmaceutical Co. Ltd., China), absolute ethanol (Sigma-Aldrich, MO, USA), formalin (Griffin & George, Leicestershire, England), potassium chloride, urethane (Sigma-Aldrich, Germany), chloralose (BDH Chemical Ltd., Poole, England), phenolphthalein, sodium hydroxide, and distilled water.

2.2 Experimental Animals

Healthy female albino mice (20 g average weight) and female Sprague-Dawley rats (180 g average weight) used in this study were obtained from the Laboratory Animal Center of the College of Medicine of the University of Lagos, Lagos, Nigeria. Experimental animals were kept in a well-ventilated room and were maintained under standard environmental conditions (23-25^{o}C, 12 h/12 h light/dark cycle). The animals had free access to standard rodent diet (Livestock Feeds Plc., Lagos, Nigeria) and water. All the animals were acclimatized for one week prior to the commencement of experimental procedures. The experiments were performed between 9.00 am and 6.00 pm on assigned days. The procedures were carried out in compliance with the United States National Academy of Sciences Guide for the Care and Use of Laboratory Animals (Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011) and the provisions of the Experimentation Ethics Committee on Animal Use of the College of Medicine, University of Lagos, Lagos, Nigeria.

2.3 Plant Material

The fresh seeds of *E. gigas* were obtained from local herb sellers at Mushin market in Mushin Local Government Area, Lagos State, Nigeria. The plant material was validated based on an existing identification and authentication done by Mr. O.O. Oyebanji of the Department of Botany and Microbiology, Faculty of Science, University of Lagos, Lagos, Nigeria. The voucher specimen (LUH 5595) was deposited in the institutional herbarium for reference purpose.

2.4 Extraction

The dark brown seeds of *E. gigas* were broken and the inner whitish flesh was removed and pulverized using a blender. Three hundred and fifty grams (350 g) of the powdered plant material was macerated in 2.5 L of distilled water for 48 h in the refrigerator with intermittent agitation to enhance extraction. Thereafter, the extract was decanted and filtered using Whatman (1.0) filter paper. The residue was re-macerated in 2.5 L of distilled water (× 2) to ensure exhaustive extraction. The filtrate was evaporated to dryness in a lab drying oven at 40 °C. A sticky, dark brown solid extract with sweet coffee-like smell and bitter taste was obtained with a yield of 66.74%. The solid extract showed hygroscopic properties in readily absorbing moisture on exposure to air. It produced foams when dissolved in water and shaken. The extract was put in sample bottles and stored in the refrigerator at 4 °C. When required, a specific amount of the extract was weighed out and dissolved in distilled water to give the desired concentration for administration to experimental animals. The doses of the extract used in this study (50, 100, 200 and 400 mg/kg) were based on results of preliminary investigations and oral acute toxicity test.

2.5 Determination of Total Flavonoids, Proanthocyanidins and Phenolic Content Twenty-five (25) g of *E. gigas* aqueous extract was dissolved in 25 ml of methanol to give a solution concentration of 1 mg/mL. In order to aid the dissolvability of the extract, sonication of the solution was carried out using Olympus KS-2 Ultrasonic bath. The solution was then filtered

using Whatman filter paper 1.0. The filtrate was used for the phytochemical analysis. The determination of total flavonoids, proanthocyanidins and phenolic content was done as reported by Sofidiya et al, (2009).

2.6 Acute Toxicity Test

The acute toxicity of the extract was determined using the OECD guidelines for the testing of chemicals; acute oral toxicity- limit test at 5000 mg/kg (OECD, 2001). Five (5) albino mice used for the procedure were fasted for 12 h prior to the test. The extract was administered orally at the dose of 5000 mg/kg. The control group received distilled water at the volume corresponding to the highest volume of extract administered to the test animal group. The animals were observed closely for behavioural changes and toxic/lethal symptoms for 2 h post-treatment (Akindele et al, 2015). Mortality within 24 h was recorded and the animals were continually observed for 14 days after administration for signs of delayed toxicity.

2.7 Antiulcer Activity Evaluation

2.7.1 Ethanol-Induced Ulcer (EIU) Test

Animals were fasted for 24 h prior to the experiment and randomly allotted into six groups of five rats each. The animals were pre-treated twice daily for 5 days as follows:

Group 1: Distilled water (10 ml/kg, *p.o.*).

Group 2: *E. gigas* aq. seed extract (50 mg/kg *p.o.*)

Group 3: *E. gigas* aq. seed extract (100 mg/kg *p.o.*)

Group 4: *E. gigas* aq. seed extract (200 mg/kg *p.o.*)

Group 5: *E. gigas* aq. seed extract (400 mg/kg *p.o.*)

Group 6: Misoprostol (a prostaglandin analogue with gastric cytoprotective activity; 50 µg/kg *p.o.*)

The rats were fasted for 24 h into the 6th day. Gastric ulcer was induced in the rats by administration of absolute ethanol (1 ml/200 g *p.o.*) (Hollander et al, 1985). After 1 h, the animals were sacrificed by cervical dislocation, after being anaesthetized with 1 ml/200 g (*i.p.*) solution of 25% urethane and 1% chloralose, and the stomach was isolated and incised along the greater curvature. The gastric lumen was rinsed with normal saline and examined for ulcers. Ulcer score was determined using the Magistreni scale (Magistretti et al, 1988). The tissues were then fixed in 10% formol-saline for histopathological assessment.

2.7.2 Pylorus Ligation-Induced Ulcer (PLIU) Test

The same treatment schedule for the ethanol-induced ulcer test was adopted except that cimetidine (a histamine H_2-receptor antagonist that inhibits stomach acid secretion) 100 mg/kg was used as positive control. The rats were fasted for 24 h into the 6th day and were anaesthetized using 25% urethane and 1% chloralose (*i.p.*) at a dose of 1 ml of mixture/200 g weight. Tracheotomy was done to remove bronchial secretion and the abdomen was opened, and pylorus ligation done without causing any damage to the blood supply. The stomach was replaced carefully and the abdominal wall was closed in two layers with a moist swab of normal saline. After 4 h, each stomach was dissected out and cut open along the greater curvature. Ulcer was scored using the Magistreni scale. The volume of gastric juice collected from each rat was determined using a measuring cylinder and the pH was thereafter measured. The centrifuged gastric juice from each pylorus-ligated rat was titrated against 0.01 N NaOH using phenolphthalein indicator and the pH was determined using a research ionalyzer (Orion, Beverly, MA, USA). All measurements were done in triplicate. Titratable acidity was determined and expressed as mEq/ml (Segawa et al, 1994; Kakub and Gulfraz, 2007).

2.7.3 Cold Restraint Stress-Induced Ulcer (CRSIU) Test

The same treatment schedule for the ethanol-induced ulcer test was adopted. Upon 24 h fasting from the 5th to the 6th day, rats were immobilized by strapping the fore and hind limbs on a flat receptive platform, and kept at a temperature of 4-6 °C for 2 h (Gupta et al, 1985). Thereafter, each rat was sacrificed as previously described above and the stomach was incised along the greater curvature. The lumen was rinsed with normal saline, examined and scored for ulcer using the Magistreni scale.

2.7.4 Ulcer Scoring Scale and Calculation of Ulcer Index and Inhibition (%)

Ulcer Scoring Scale

0 = no lesion

0.5 = hemorrhage

1 = 1-3 small lesions (10 mm length)

2 = 1-3 large lesions (10 mm length)

3 = 1-3 thickened lesions

4 = more than 3 small lesions

5 = more than 3 large lesions

6 = more than 3 thickened lesions (Magistretti et al, 1988)

Calculation of Ulcer Index and Inhibition (%)

$$UI = ADU \times (\%RU/100)$$

Where ADU is the average degree of ulceration for each group (mean ulcer score) and %RU is the percentage of rats with ulceration (Thuillier et al, 1968).

$$\text{Inhibition } (\%) = ([UI_C - UI_T]/UI_C) \times 100$$

Where UI_C is the ulcer index for the control group and UI_T is the ulcer index for the treatment group.

2.8 Statistical Analysis

The results obtained in this study were expressed as mean ± S.E.M. (standard error of mean). Data were analyzed using one-way ANOVA using Dunnett's post-hoc test (GraphPad Prism 6, GraphPad Software Inc., CA, USA) and Student's t-test (unpaired; two-tailed) for independent comparisons where applicable). Values were considered significant at $p < 0.05$.

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3. Results

3.1 Determination of Total Flavonoids, Proanthocyanidins and Phenolic Content

Flavonoids, proanthocyanidins, and phenolic compounds were present in the aqueous seed extract of *E. gigas*. The total flavonoids, proanthocyanidins and phenolic content were estimated to be 9.898 mg/g, 8.346 mg/g, and 105.476 mg/g of the extract, respectively.

3.2 Acute Toxicity Test

The aqueous seed extract of *E. gigas* administered orally to mice at the dose of 5000 mg/kg neither caused mortality nor produced toxic/lethal signs.

3.3 Ethanol-Induced Ulcer Test

Ulcer Score and Index

E. gigas (50-400 mg/kg) and misoprostol (50 µg/kg) produced significant reduction ($p < 0.05$-0.001) in ulcer scores compared with control (10.80 ± 0.80).

The greatest reduction was produced at the extract dose of 100 mg/kg (ulcer score 2.30 ± 0.99; 87.22% inhibition). This effect was comparable and not significantly different ($p > 0.05$) from that elicited by misoprostol (ulcer score 3.00 ± 1.31; 83.33% inhibition) (**Table 1**).

Table 1: Effect of *E. gigas* on ethanol-induced ulcer in rats

Treatment and dose	Ulcer score	Ulcer index	Inhibition (%)
Distilled water 10 ml/kg	10.80 ± 0.80	10.80	-
E. gigas 50 mg/kg	5.40 ± 0.87*	5.40	50.00
E. gigas 100 mg/kg	2.30 ± 0.99***	1.38	87.22
E. gigas 200 mg/kg	4.70 ± 1.51*	4.70	56.48
E. gigas 400 mg/kg	3.30 ± 1.38**	2.64	75.56
Misoprostol 50 µg/kg	3.00 ± 1.31***	1.80	83.33

*Values are expressed as Mean ± S.E.M. (n = 5). *p < 0.05, **p < 0.01, ***p < 0.001 vs. distilled water (one-way ANOVA with Dunnett's post-hoc test).*

Table 2: Effect of *E. gigas* on pylorus ligation-induced ulcer in rats

Treatment and dose	Ulcer score	Ulcer index	Inhibition (%)
Distilled water 10 ml/kg	6.30 ± 0.70	6.30	-
E. gigas 50 mg/kg	4.60 ± 1.18	3.68	41.59
E. gigas 100 mg/kg	3.40 ± 0.94#	2.72	56.83
E. gigas 200 mg/kg	2.90 ± 0.83#	2.32	63.17
E. gigas 400 mg/kg	3.50 ± 0.92#	3.50	44.44
Cimetidine 100 mg/kg	2.60 ± 0.93*,#	2.08	66.98

*Values are expressed as Mean ± S.E.M. (n = 5). *,#p < 0.05 vs. distilled water (*one-way ANOVA with Dunnett's post-hoc test; #Student's t-test, unpaired and two-tailed).*

Table 3: Effect of *E. gigas* on gastric content volume, pH and titratable acidity in pylorus ligation-induced ulcer in rats

Treatment and dose	Volume of gastric content (mL/4 h)	Reduction in volume (%)	pH	Titratable acidity (mEq/mL)
Distilled water 10 ml/kg	2.32 ± 0.33	-	2.98 ± 0.18	2.17 ± 0.36
E. gigas 50 mg/kg	0.24 ± 0.07***	89.66	3.26 ± 0.13	1.75 ± 0.42
E. gigas 100 mg/kg	1.40 ± 0.43	39.66	3.28 ± 0.02	1.18 ± 0.18
E. gigas 200 mg/kg	1.06 ± 0.22*	54.31	3.08 ± 0.15	1.32 ± 0.31
E. gigas 400 mg/kg	1.26 ± 0.34	45.69	2.98 ± 0.13	1.76 ± 0.34
Cimetidine 100 mg/kg	1.02 ± 0.16*	56.03	3.70 ± 0.20*	0.97 ± 0.18

*Values are expressed as Mean ± S.E.M. (n = 5). *p < 0.05, ***p < 0.001 vs. distilled water (one-way ANOVA with Dunnett's post-hoc test).*

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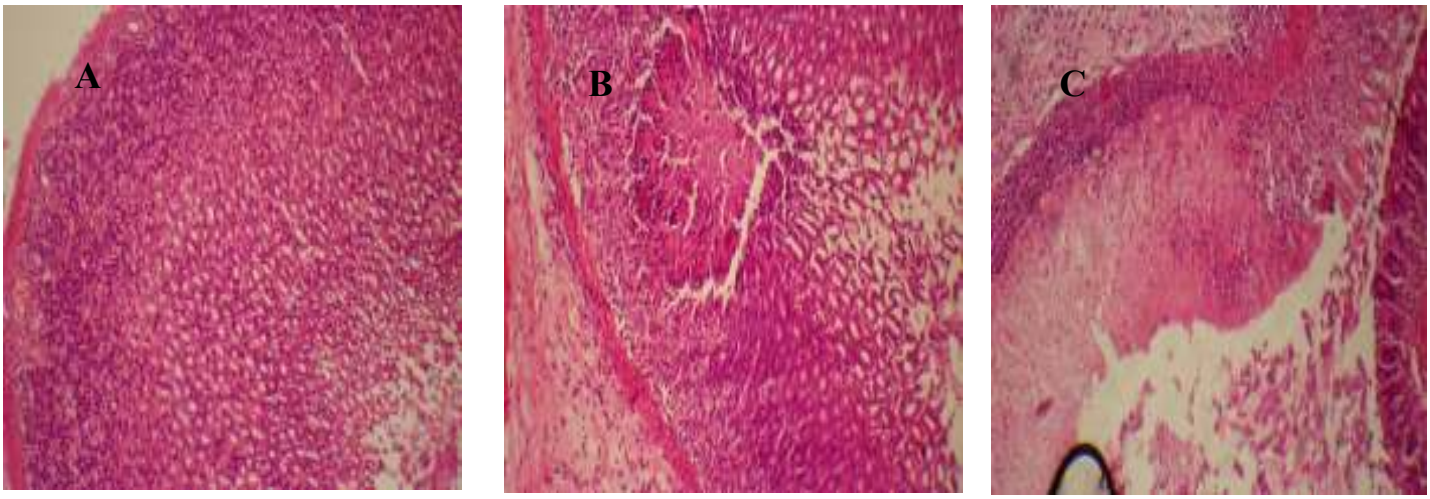

Figure 1: Photomicrographs of rat gastric representative tissue for the ethanol-induced ulcer test treated with distilled water 10 ml/kg (**A**; showing full thickness mucosal necrosis/ulceration and sub-mucosal inflammation); *E. gigas* 100 mg/kg (**B**; showing superficial mucosal necrosis/erosion); and misoprostol 50 µg/kg (**C**; showing normal histologic section with gastric mucosa without areas of necrosis or inflammation) (× 100).

Table 4: Effect of *E. gigas* on cold restraint stress-induced ulcer in rats

Treatment and dose	Ulcer score	Ulcer index	Inhibition (%)
Distilled water 10 ml/kg	4.20 ± 0.64	4.20	-
E. gigas 50 mg/kg	0.80 ± 0.49**	0.32	92.38
E. gigas 100 mg/kg	3.00 ± 0.76	2.40	42.86
E. gigas 200 mg/kg	1.30 ± 0.80*	0.52	87.62
E. gigas 400 mg/kg	1.00 ± 0.63**	0.40	90.48
Misoprostol 50 µg/kg	1.40 ± 0.60*	0.84	80.00

*Values are expressed as Mean ± S.E.M. (n = 5). *p < 0.05 vs. distilled water (one-way ANOVA with Dunnett's post-hoc test).*

Histopathological Assessment of Gastric Tissue

As shown in **Figure 1**, photomicrographs of rat gastric representative tissue for the ethanol-induced ulcer test revealed full thickness mucosal necrosis/ulceration and sub-mucosal inflammation for distilled water 10 mL/kg, superficial mucosal necrosis/erosion for *E. gigas* 100 mg/kg, and normal histologic section with gastric mucosa without areas of necrosis or inflammation for misoprostol 50 µg/kg.

3.4 Pylorus Ligation-Induced Ulcer Test

Ulcer Score and Index

The extract (100-400 mg/kg) caused significant reduction ($p < 0.05$) in the ulcer score compared with control (6.30 ± 0.70). The inhibition of ulcer development was highest at the dose of 200 mg/kg (ulcer score 2.90 ± 0.83; 63.17%). Cimetidine produced significant reduction ($p < 0.05$) in the ulcer score (2.60 ± 0.93; 66.98%) relative to control. The effect of cimetidine was not significantly different ($p > 0.05$) from the effects of *E. gigas* at all doses tested (**Table 2**).

Gastric Content Volume, pH and Titratable Acidity

E. gigas at 50 and 200 mg/kg, and cimetidine 100 mg/kg (0.24 ± 0.07, 1.06 ± 0.22 and 1.02 ± 0.16 ml/4 h, respectively) significantly reduced ($p < 0.01$, 0.001) the volume of gastric content relative to control (2.32 ± 0.33 ml/4 h) with reduction in volume (%) values of 89.66, 54.31 and 56.03%, respectively.

The effect of the extract in reducing the gastric content volume at 50 mg/kg was greater than that elicited by cimetidine, while the value for *E. gigas* at 200 mg/kg was comparable to that of the standard drug. The extract at all doses used did not significantly alter ($p > 0.05$) the pH and titratable acidity of the gastric content compared with control. Cimetidine significantly increased ($p < 0.05$) the pH but not titratable acidity value relative to control (**Table 3**).

3.5 Cold Restraint Stress-Induced Ulcer Test

3.5.1 Ulcer Score and Index

E. gigas at doses of 50, 200 and 400 mg/kg caused significant reduction ($p < 0.05$, 0.01) in the ulcer score, with the greatest effect produced at the dose of 50 mg/kg (0.80 ± 0.49; 92.38% inhibition), relative to control (4.20 ± 0.64). However, the effect of the extract at the dose of 50 mg/kg was comparable and not significantly different ($p > 0.05$) from the result at other extract doses and that elicited by misoprostol (1.40 ± 0.60; 80% inhibition) (**Table 4**).

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4. Discussion

Peptic ulcers, with gastric and duodenal ulcers being the most common, result from an imbalance between aggressive (hydrochloric acid, pepsin, refluxed bile, leukotrienes, and reactive oxygen species) and defensive (mucus-bicarbonate barrier, prostaglandins, mucosal blood flow, cell renewal and migration, non-enzymatic and enzymatic antioxidants, and some growth factors) factors (Amandeep et al, 2012). In this study, the aqueous seed extract of *E. gigas* was evaluated for gastroprotective effects using the ethanol-, pylorus ligation-, and cold restraint stress-induced ulcer tests in rats.

Ethanol is a corrosive agent that penetrates the gastric mucosa due to its ability to solubilize the protective mucous and expose the mucosa to the proteolytic and hydrolytic activity of hydrochloric acid secreted from the parietal cells of the stomach and pepsin (Soll, 1990; Surendra, 1999). It has been reported that the incidence of ethanol-induced ulcer is predominant in the glandular part of the stomach and ethanol stimulate the formation of leukotriene C4 (LTC4), mast cell secretory products, and reactive oxygen (Salim, 1990). In this model, *E. gigas* (50-400 mg/kg) produced significant reduction in ulcer development with the most prominent effect produced at the dose of 100 mg/kg (87.22% inhibition). This effect was comparable to that elicited by misoprostol (83.33% inhibition). The biphasic inhibitory action of *E. gigas* (observation of greatest effects at 100 and 400 mg/kg with peak effect at the lower dose) may be explained by the concept of Hormesis. Hormesis is a biphasic dose response to an agent characterized by low dose stimulation or beneficial effect and a high dose inhibitory or toxic effect (Mattson, 2008). Photomicrographs of rat gastric representative tissue for this test revealed superficial mucosal erosion for the extract at the dose of 100 mg/kg compared with full thickness mucosal ulceration for the negative control. The findings in the ethanol-induced ulcer test suggest that the extract possibly reduced the ability of the ulcerogen to solubilize the protective gastric mucous layer- a direct cytoprotective effect. This effect may be due to increased mucus secretion (a possibility that needs to be further investigated) or enhanced mucosal resistance that prevents damage of the gastric mucosa.

The digestive effect of accumulated gastric juice and interference of gastric blood circulation are responsible for the induction of ulceration by pylorus ligation (Khushtar et al, 2009; Patel et al, 2000). According to Sairam et al, (2002), pylorus ligation-induced ulcers are due to the auto-digestion of the gastric mucosa and breakdown of the gastric mucosal barrier. In this model, the extract (100-400 mg/kg) caused significant reduction in ulcer development with peak inhibitory effect at the dose of 200 mg/kg (63.17%). The biphasic inhibitory effect of the extract in this test may also be attributed to Hormesis. The maximal effect of the extract was comparable to that of 100 mg/kg cimetidine (a histamine H_2-receptor antagonist that inhibits stomach acid secretion; 66.98% inhibition). *E. gigas* at 50 and 200 mg/kg, and cimetidine significantly reduced the volume of gastric content with reduction in volume (%) values of 89.66, 54.31 and 56.03%, respectively. However, *E. gigas* did not significantly change the pH and titratable acidity of the gastric content. The findings in this test suggests that *E. gigas* possesses anti-secretory property. It is however not clear if this effect is general or specific to certain gastric secretory cells.

Ulceration in the cold restraint stress model is mainly due to increased acid secretion and generation of free radicals which cause oxidative gastric mucosal damage (Vijayakumar et al, 2011). *E. gigas* (50, 200 and 400 mg/kg) caused significant reduction in ulcer development with the most pronounced effect elicited at the dose of 50 mg/kg (92.38% inhibition). As with the other two models, the greatest ulcer development inhibitory effect was observed at the lower dose, a phenomenon which had earlier been ascribed to the concept of Hormesis. The peak effect of the extract in this model was comparable to that produced by misoprostol (80% inhibition). The action of *E. gigas* in the cold restraint stress test may be due to its anti-secretory property, as established in the pylorus ligation model.

In respect of acute toxicity, oral administration of *E. gigas* (5000 mg/kg) did not produce any mortality or symptoms of toxicity in the treated mice. 14 days post-acute oral administration, no mortality and significant change in behavior of the animals was observed. Loomis and Hayes (1996) described the classification of some chemical agents into categories of toxicity in which the dose of 5000 mg/kg was categorized as practically non-toxic. This suggests that the extract is relatively safe administered orally.

Phytochemical analysis of *E. gigas* revealed the presence of flavonoids, proanthocyanidins and phenolic compounds. These phytoconstituents are known antioxidants with free radicals scavenging properties. Although the extract did not cause significant change in gastric tissue antioxidant indices in the cold restraint stress model (results not shown), this possibility needs to be specifically and broadly explored. Flavonoids are known to reduce gastric ulcer formation (Suzuki et al, 1998), hence may contribute in part to the antiulcer activity of the extract observed in this study. Wittschier et al, (2007) reported that proanthocyanidins present potential prophylactic tools against specific bacterial infections including *H. pylori*, *Campylobacter jejuni* etc. As earlier mentioned, the antimicrobial activity of *E. gigas* had previously been reported (Fankam et al, 2014). In view of this fact, the proanthocyanidins content of *E. gigas* may also contribute important prophylactic effects by antagonizing the adhesive interaction of these bacteria to the gastrointestinal tract. However, this possibility needs to be further investigated.

5. Conclusion

The results obtained in this study suggest that the aqueous seed extract of *Entada gigas* possess gastroprotective effects possibly mediated via cytoprotective and anti-secretory activities and associated with the presence of flavonoids, proanthocyanidins and phenolic compounds detected. However, further studies are ongoing to isolate, elucidate and characterize the precise chemical principle(s) responsible for the established gastroprotective effects of the extract and determine the mechanism(s) of action, including possible antioxidant/radical scavenging activities. The findings in

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this study justify the use of the extract for the treatment of ulcer traditionally.

Conflict of Interest Declaration

The authors declare no conflicts of interest.

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References

Adinortey MB, Ansah C, Galyuon I, Nyarko A (2013). In vivo models used for evaluation of potential antigastroduodenal ulcer agents. *Ulcers*, **2013**: 796405, http://dx.doi.org/10.1155/2013/796405

Akindele AJ, Unachukwu EG, and Osiagwu DD (2015). 90 Days toxicological assessment of hydroethanolic leaf extract of *Ipomoea asarifolia* (Desr.) Roem. and Schult. (Convolvulaceae) in rats. *J. Ethnopharmacol.* **174**: 582-594.

Amandeep K, Robin S, Ramica S, and Sunil K (2012). Peptic ulcer: a review of etiology and pathogenesis. *Int. Res. J. Pharm.* **3**: 34-38.

Andrade SF, Antoniolli D, Comunello E, Cardoso LGV, Carvalho JCT, and Bastos JK (2006). Antiulcerogenic activity of crude extract, fractions and populnoic acid isolated from *Austroplenckia populnea* (Celastraceae). *Zeistschrift Fur Naturforschung C*, **61**: 329-333.

Begum N, Mayuren C, Balaji N, Chinnapa RY, and Aravind KK (2008). Evaluation of hepatoprotective activity of aqueous extract of *Curcuma longa* in carbon tetrachloride induced hepatotoxicity in rats. *Adv. Pharmacol. Toxicol.* **9**: 33-36.

Committee for the Update of the Guide for the Care and Use of Laboratory Animals (2011). *Guide for the Care and Use of Laboratory Animals* (Eighth Edition). National Academy of Sciences. Washington DC: National Academies Press.

Dahanukar SA, Kulkarni RA, and Rege NN (2000). Pharmacology of medicinal plants and natural products. *Indian J. Pharmacol.* **32**: 81-118.

Dashputre NL and Naikwade NS (2011). Evaluation of anti-ulcer activity of methanolic extract of *Abutilon indicum* Linn leaves in experimental rats. *Int. J. Pharmaceut. Sci. Drug Res.* **3**: 97-100

Falk GW (2001). *Cecil Essentials of Medicine* (5th Edition). Edinburgh: WB Saunders Company, pp 334-343.

Fankam AG, Kuiate JR, and Kuete V (2014). Antibacterial activities of *Beilschmiedia obscura* and six other Cameroonian medicinal plants against multi-drug resistant Gram-negative phenotypes. *BMC Complement. Altern. Med.* **14**: 241, doi: 10.1186/1472-6882-14-241

Fasinu PS, Bouic PJ, and Rosenkranz B (2012). An overview of the evidence and mechanisms of herb-drug interactions. *Front. Pharmacol.* **3**: 69, doi: 10.3389/fphar.2012.00069

Gunn CR and Dennis JV (1976). *World Guide to Tropical Drift Seeds and Fruits*. A Demeter Press Book. Quadrangle/The New York Times Book Co., New York.

Gupta MB, Nath R, Gupta GP, and Bhargava KP (1985). A study of the antiulcer activity of diazepam and other tranquillosedatives in albino rats. *Clin. Exp. Pharmacol. Physiol.* **12**: 61-66.

Hollander D, Tarnawski A, Krause WJ, and Gergely H (1985). Protective effect of sucralfate against alcohol-induced gastric mucosal injury in the rat. *Gastroenterology*, **88**: 366-374.

Kakub G and Gulfraz M (2007). Cytoprotective effects of *Bergenia ciliate* Sternb extract on gastric ulcer in rats. *Phytother. Res.* **21**: 1217-1220.

Khushtar M, Kumar V, Javed K, and Bhandari U (2009). Protective effect of ginger oil on aspirin and pylorus ligation-induced gastric ulcer model in rats. *Indian J. Pharm. Sci.* **71**: 554-558.

Loomis TA and Hayes AW (1996). *Essentials of Toxicology* (4th edition). Academic Press Limited, London, pp 33-46.

Magistretti MJ, Conti M, and Cristoni A. Antiulcer activity of an anthocyanidin from *Vaccinum myritillus*. *Arzneimittelforschung*, **38**: 686-690.

Mattson MP (2008). Hormesis defined. *Ageing Res. Rev.* **7**: 1-7.

Organization for Economic Corporation and Development (OECD) (2001). OECD guideline for testing of chemicals- acute oral toxicity. http://www.oecd.org/chemicalsafety/risk-assessment/1948370.pdf, accessed on 20th January, 2015.

Patel AV, Santani DD, and Goel RK (2000). Antiulcer activity and the mechanism of action of magaldrate in gastric ulceration models of rat. *Indian J. Physiol. Pharmacol.* **44**: 350-354.

Sairam K, Rao ChV, Babu MD, Kumar KV, Agrawal VK, and K Goel RK (2002). Antiulcerogenic effect of methanolic extract of *Emblica officinalis*: an experimental study. *J. Ethnopharmacol.* **82**: 1-9.

Sairam K, Rao CV, and Goel RK (2001). Effect of *Centella asiatica* Linn on physical and chemical factors induced gastric ulceration and secretion in rats. *Indian J. Exp. Biol.* **39**: 137-142.

Salim AS (1990). Removing oxygen-derived free radicals stimulates healing of ethanol-induced erosive gastritis in the rat. *Digestion*, **47**: 24-28.

Sánchez-Mendoza ME, Reyes-Ramírez A, Antonio LC, Jiménez LM, Rodríguez-Silverio J, Arrieta J (2011). Bioassay-guided isolation of an anti-ulcer compound, tagitinin C, from *Tithonia diversifolia*: role of nitric oxide, prostaglandins and sulfhydryls. *Molecules*, **16**: 665-674.

Segawa K, Arisawa T, Niwa Y, Kato T, Tsukamoto Y, Goto H, Hayakawa T, and Nakazawa S (1994). The relationship between titrated acidity (mEq/L) and pH of human gastric juice: a study based on the data estimated by pH-meter. *Nihon Shokakibyo Gakkai Zasshi*, **91**: 849-853.

Sofidiya MO, Odukoya OA, Afolayan AJ, and Familoni OB (2009). Phenolic contents, antioxidant and antibacterial activities of *Hymenocardia acida*. *Nat. Prod. Res.* **23**: 168-177.

Soll AH (1990). Pathogenesis of peptic ulcer and implications for therapy. *N. Engl. J. Med.* **322**: 909-916.

Srinivas TL, Lakshmi SM, Shama SN, Reddy GK, and Prasanna KR (2013). Medicinal plants as anti-ulcer agents. *J. Pharmacogn. Phytochem.* **2**: 91-97.

Surendra S (1999). Evaluation of gastric anti-ulcer activity of fixed oils of tusil and possible mechanisms. *Indian J. Exp. Biol.* **36**: 253-257.

Suzuki Y, Ishihara M, Segemi T, and Ito M (1998). Anti-ulcer effects of antioxidants, quercetin, alpha-tocopherol, nifedipine and tetracycline in rats. *Jpn. J. Pharmacol.* **78**: 435-441.

Thuillier J, Bessin P, Geoffroy FA, Godfroid J, Chimic J (1968). Pharmacologic de la clofezone. *Chim. Ter.* **3**: 53-67.

Vijayakumar M, Eswaran MB, Ojha SK, Rao ChV, and Rawat AK (2011). Antiulcer activity of hydroalcohol extract of *Momordica dioica* roxb. Fruit. *Indian J. Pharm. Sci.* **73**: 572-577.

Wills RBH, Bone K, and Morgan M (2000). Herbal products: active constituents, modes of action and quality control. *Nutr. Res. Rev.* **13**: 47-77.

Wittschier N, Lehgsfield C, Vorthemia S, Stratmann U, Ernst JF, Verspohl EJ, and Hensel A (2007). Large molecules as anti-adhesive compounds against pathogens. *J. Pharm. Pharmacol.* **59**: 777-786.

Zdanowicz MM (2002). *Peptic ulcer*. In: Essentials of Pathophysiology for Pharmacy. CRC Press Pharmacy Education Series, p 170.

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