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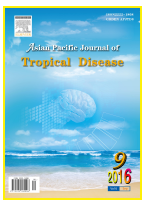

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Preliminary studies on the anti-ulcer potentials of *Vitex doniana* crude extracts on experimental rat model of ethanol induced gastric ulcer

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ABSTRACT

Objective: To investigate antiulcerogenic potentials of the crude extracts of *Vitex doniana* against ethanol induced gastric ulcer.
Methods: Twenty four rats divided into 6 groups (I–VI) of 4 animals each were used. The animals were fasted for 24 h at the end of which Group I received a single dose of 10 mL/kg of saline; Group II received a single dose of 100 mg/kg cimetidine intramuscularly; Groups III and IV received single doses of 200 mg/kg and 400 mg/kg body weight of the methanol extract while Groups V and VI received 200 mg/kg and 400 mg/kg body weight of the aqueous extract respectively. After 30 min, each animal received 1 mL/kg body weight of absolute ethanol orally and was sacrificed 1 h later under chloroform anesthesia. The animals' stomachs were excised and the ulcers were counted and scored with the aid of a magnifying lens (×10). The mean ulcer index and ulcer preventive index were calculated for each group.
Results: A statistically significant decrease ($P < 0.05$) in mean ulcer index was observed in Groups II, IV and V compared to that of control Group I. Calculated percentage ulcer preventive index showed a 97%, 53%, 82%, 82% and 22% percentage preventive index for Groups II, II, IV, IV and VI respectively.
Conclusions: Crude extracts of *Vitex doniana* possess antiulcer properties against ethanol induced gastric ulcers.

1. Introduction

Medicinal plants are the source of many modern medicines and are used on a daily basis for the treatment of various ailments worldwide[1]. These plants are important in the treatment of diseases and remain the chief alternative for most people[2]. Plant extracts have become an important source of new drugs which have shown promising results for the treatment of gastric ulcer[3] and diarrhea[4]. Approximately 70% of people in the developing world use medicinal plants for treating diseases with most people depending solely on them for their basic health care needs[1,5].

Vitex doniana (*V. doniana*), family Verbenaceae, commonly called "black plum" and "ucha kiri" in Igbo, is widely distributed in the eastern and western parts of Nigeria[6]. In Nigeria, various parts of the plant are used traditionally for managing and treating several disorders including rheumatism, hypertension, cancer, and inflammatory diseases[7]. Various parts of the plant are used to treat some gastrointestinal disorders like diarrhea, hemorrhoids, constipation, ulcers and dysentery[8].

Gastric ulcers constitute a major class of gastrointestinal disorder attracting global attention in health care[9]. About 4 million people are affected by gastric ulcers worldwide. Out of this, about 10% to 20% would develop complications[10]. Major predisposing factors for gastric ulcer include severe stress, *Helicobacter pylori* infection, alcohol consumption, and non-steroidal anti-inflammatory drugs[11].The gastric mucosa is also frequently exposed to potentially harmful agents such as food ingredients, acid, bile acids, pepsin, bacterial products, and drugs. These agents have been identified as important factors in the development of gastric ulcers, such as an increased pepsin and

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gastric acid secretion, decreased gastric perfusion, suppressed production of prostaglandins, inhibition of mucosal cell growth and proliferation, and altered gastric mobility. Studies also suggest that the generation of oxygen free radicals plays a major role in the general pathogenesis of gastric ulcers. In cases of ethanol induced gastric ulcers, lipid peroxidation is an important aspect of the pathogenesis. Treatment strategy for gastric ulcer is targeted at the control of *Helicobacter pylori* as well as H^+/K^+-ATPase, acid secretion and a restoration of damaged mucosa and cessation of inflammation[11,12].

Although various other medicinal properties of *V. doniana* have been investigated, there is a paucity of information and documented scientific report on its antiulcerogenic potentials. This research was therefore designed to explore the use of the *V. doniana* leaves as a potential remedy for gastrointestinal ulcers.

2. Materials and methods

2.1. Plant material

Fresh tender leaves of *V. doniana* were collected from the University of Nigeria Enugu Campus environs during the months of July and August, 2013. The leaves were duly identified and confirmed to be *V. doniana* by botanists at the Department of Plant Science and Biotechnology, University of Nigeria Nsukka. A voucher specimen was deposited at the herbarium (Enugu, Nigeria: Enugu: Udi, Latilo FHI 27617)[13]. The leaves were dried under shade and ground into powder using a gasoline powered grinding machine.

For the aqueous extract, 2 500 mL of water was added to 350 g of the *V. doniana* powder and was homogenized using a wooden stirrer. The homogenate was sieved using a muslin cloth and the obtained filtrate was stored in the refrigerator at 2–8 ℃ until required for use.

For the crude methanol extract, 1 500 g of the powder were soaked in 8 L of 80% methanol for 72 h. The mixture was constantly agitated during the extraction process. After 72 h, the mixture was filtered using Whatman filter paper and the filtrate was allowed to evaporate. The residue obtained was stored in a refrigerator until required. The extractive values of both extracts were determined. The methanol extract was reconstituted by initially dissolving in 3% Tween 80 and making up to 100 mL with the same solvent.

2.2. Experimental animals

Male Albino Wistar rats weighing 90–150 g were obtained from the Animal House, College of Medicine, University of Nigeria, Enugu campus. They were housed under standard conditions of temperature [(27 ± 2) ℃] and a 12 h light, 12 h dark cycle. The animals were housed in groups in metallic cages and were fed on standard commercial rat feed (Guinea Feed®, Enugu, Nigeria) and clean water *ad libitum*. They were allowed for a period of two weeks for acclimatization, before the commencement of the studies.

2.3. Acute toxicity testing

A modification of the method described by Lorke[14] for the determination of LD_{50} was used. The experiment was carried out in two phases using adult Albino mice (weighing 20–25 g). In the first phase, three different doses of the plant extracts were administered to three groups of three animals each. Groups A, B and C received 10, 100 and 1 000 mg/kg body weight of the extracts respectively after an overnight fast. They were observed for a 24 h period for signs of acute toxicity such as dullness, depression, diarrhoea and death.

From the results in phase one, the second phase was performed using doses of 1 500, 2 500 and 3 500 mg/kg body weight respectively for three groups of four animals per group. This procedure was done for both the aqueous and methanolic extracts.

2.4. Induction of gastric ulcer using absolute ethanol

Ninety-five percent ethanol was used to induce gastric ulcers using a modification of the method of Robert[15], as described by Choudhary *et al.*[9].

Briefly, 24 Albino Wistar rats were divided into six groups of four animals per group. The animals were fasted for 24 h prior to the start of administration but had with free access to water. At the end of the fasting period, Group I (negative control) received 10 mL/kg of saline. Animals in Group II (positive control) were given 100 mg/kg cimetidine. Group III and IV received 200 mg/kg and 400 mg/kg methanolic extract of *V. doniana* while Groups V and VI received 200 mg/kg and 400 mg/kg body weight of the aqueous extract of *V. doniana* respectively. A single dose of the extracts was administered via the oral route using an orogastric tube while a single dose of cimetidine was administered via the intramuscular route using a hypodermic needle.

Thirty minutes after the administration, each of the animals were given 1 mL/kg body weight of 95% ethanol orally. After 1 h the animals were sacrificed under chloroform anesthesia and their stomachs were removed, rinsed in saline and opened along the greater curvature. The ulcers were viewed and counted with the aid of a magnifying lens (×10). The ulcerative lesion index was calculated as follows: ulcerative lesion < 1 mm = 1; ulcerative lesion > 1 mm < 2 mm = 2; ulcerative lesion > 2 mm = 3.

The sum of the scores was divided by 10 to derive the ulcer index for each rat. The effectiveness of the extract and drug was calculated using the formula:

Ulcer preventive index (%) = Ulcer index of control - Ulcer index of treated/Ulcer index of control × 100[3,16].

2.5. Tissue histology

The organs excised from the sacrificed rats were subjected to

histological processing: dehydration, clearing, wax impregnation, and embedding. And 5 µm-thick sections of the tissue were obtained using the rotary microtome (Leitz 1520 Rotary Microtome, Leica Biosystems, Nussloch Germany). The tissue sections were stained according to the haematoxylin and eosin technique as described by Baker and Silverton[17].

2.6. Microscopy and photomicrography

The stained sections were examined using a Swift® binocular microscope with an inbuilt lighting system. The sections were photographed using a Samsung® NX1000 digital camera attached to a Magnus® trinocular microscope.

2.7. Statistical analysis

Data obtained in the study was analysed using the SPSS version 20. The results were expressed where appropriate as mean ± SEM. The Dunnett's test was adopted for statistical comparison of means. Results with $P < 0.05$, 0.01 and 0.001 were considered statistically significant.

3. Results

Oral administration of absolute ethanol at a dose of 1 mL/kg produced ulcers in all treated animals. Gross examination of the excised animal stomachs showed varying degrees of haemorrhagic ulcers in the different treatment groups with the negative control (Group I) having the most severe ulcerations while the cimetidine control (Group II), Groups IV and V had the least number of ulcers (Figure 1). Histopathological examination of the excised stomachs also revealed the same pattern of severity of ulcerations observed in the gross studies (Figure 2).

Calculated mean ulcer index showed that the animals in the negative control group (Group I) had the highest ulcer index of 1.70 ± 0.17 while the positive control group (II) had the lowest (0.10 ± 0.17). Groups IV and V had similarly low ulcer indices of 0.30 ± 0.30 while Groups III and VI had relatively higher ulcer indices of 0.80 ± 0.85 and 1.33 ± 0.72 respectively. Similarly, the groups that were treated with cimetidine had the highest value for calculated ulcer preventive index (97%) followed by groups IV and VI (400 mg/kg body weight methanolic extract of *V. doniana* and 200 mg/kg body weight aqueous extract of *V. doniana* respectively) both having a ulcer preventive index of 82%. Group VI (400 mg/kg body weight aqueous extract of *V. doniana*) had the least ulcer preventive index of 22% while Group III (200 mg/kg body weight methanolic extract of *V. doniana*) had a 53% ulcer preventive index (Table 1).

Table 1
Mean ulcer index and ulcer preventive index in different treatment groups following ethanol induced gastric ulcer rats.

Groups	Treatment	Doses (mg/kg body weight)	Ulcer index[#]	*P*-value	Ulcer preventive index (%)
I	Water	1 mL/animal	1.70 ± 0.17	-	-
II	Cimetidine	100	0.10 ± 0.17[a]	0.003	97
III	MEVD	200	0.80 ± 0.85	0.065	53
IV	MEVD	400	0.30 ± 0.30[a]	0.007	82
V	AEVD	200	0.30 ± 0.52[a]	0.007	82
VI	AEVD	400	1.33 ± 0.72	0.445	22

[#]: Values are expressed as mean ± SEM; [a]: $P < 0.05$ when compared with control; MEVD: Methanolic extract of *V. doniana*; AEVD: Aqueous extract of *V. doniana*.

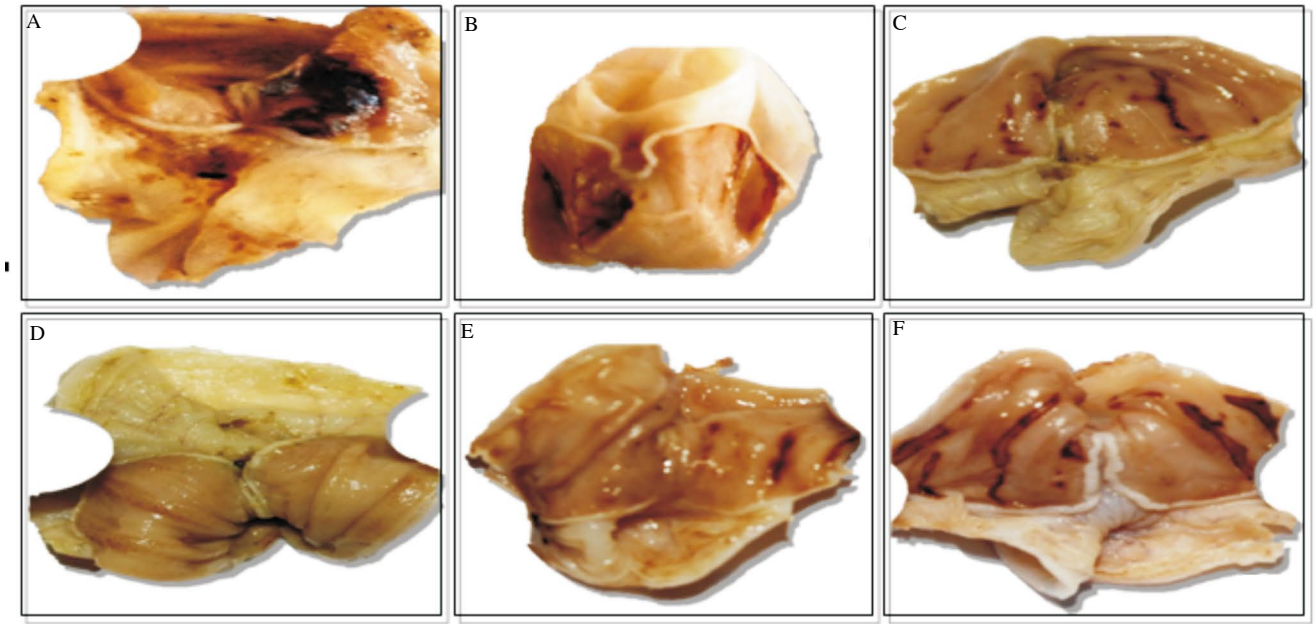

Figure 1. Macrographs showing the gross appearance of the excised stomach mucosae of rats in different treatment groups.
A: Group I (1 mL/kg absolute ethanol only) showing severe haemorrhagic ulceration of the stomach mucosa; B: Group II (100 mg/kg cimetidine) showing mild streak haemorrhagic ulcerations; C: Group III (200 mg/kg methanolic extract of *V. doniana*) showing moderately severe streak ulcers; D: Group IV (400 mg/kg methanolic extract of *V. doniana*) showing very mild spot ulcerations; E: Group V (200 mg/kg aqueous extract of *V. doniana*) showing mild ulcerations; F: Group VI (400 mg/kg aqueous extract of *V. doniana*) showing severe ulcerations of the gastric mucosa.

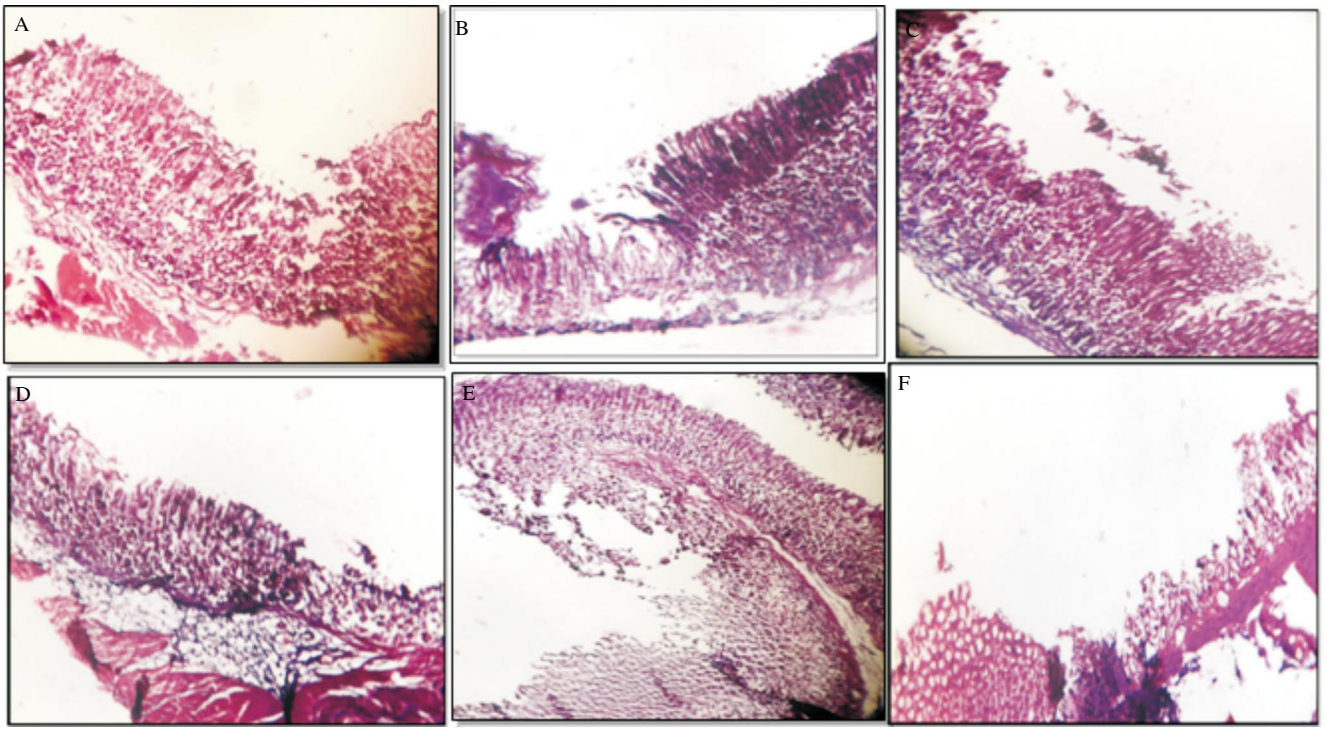

Figure 2. Micrographs of the histological sections of the stomachs of rats in the various treatment groups I–VI (A–E in the figure). Sections of all treatment groups showed varying degrees of gastric mucosal erosions, deposits of mucosal debris and infiltration of mucosae by inflammatory leucocytes.

4. Discussion

This study was designed to study the possibility of using the extracts from the plant as a readily available and safe alternative to orthodox medications for the treatment of gastric ulcers. Results of preliminary oral acute testing performed on mice revealed that both the aqueous and methanolic extracts of *V. doniana* had a LD_{50} greater than 3500 mg/kg body weight. At this dose the animals showed no visible sign of distress. This suggests that the extracts are safe for consumption since the LD_{50} is greater than 2000 mg/kg body weight[18].

Administration of a single dose of 1 mL/kg body weight of absolute ethanol produced haemorrhagic ulcer lesions in the rats was used for the study. Gross examination of the excised stomachs revealed varying degrees of ulcerations. Ethanol may cause damage to the gastric mucosa by directly acting on the gastric epithelial cells leading to lipid peroxidation. Lipid peroxidation causes oxidative cellular damage and occurs following the interaction between hydroxyl radicals and cell membranes leading to the production of extremely reactive lipid-peroxide-derived free radicals[19]. By these mechanisms, ethanol can cause the death of gastric mucosal cells by inducing intracellular oxidative stress[20].

Pretreatment of the rats with cimetidine (a standard antiulcer drug) or the extracts 1 h prior to ulcer induction by ethanol produced significant protection of the gastric mucosal walls against haemorrhagic ulcerations caused by ethanol administration. This protection was seen by the statistically significant decrease ($P < 0.05$) in mean ulcer index of the animals in Groups IV and V which received 400 mg/kg body weight of methanolic extract of *V. doniana* and 200 mg/kg body weight of aqueous extract of *V. doniana* respectively when compared with that of Group I which received only ethanol without any pretreatment. Calculated percentage ulcer protective indices for the treatment groups (III to VI) showed that the extracts afforded some degree of protection to the animals against ethanol-induced gastric ulceration. The ulcer preventive index of the methanolic extract at a dose of 400 mg/kg body weight and that of the aqueous extract at 200 mg/kg body weight was seen to be comparable to the ulcer preventive index of cimetidine while the aqueous extract at a dose of 400 mg/kg body weight showed the least protection.

Extracts of *V. doniana* have been shown to have flavonoids, saponins and tannins among many other active phytochemicals[7,21]. Flavonoids and tannins are among the active compounds in plants which offer protection against gastric lesions by acting as gastric protective factors[2].

Flavonoids are naturally occurring phenolic compounds with low molecular weight shown to exhibit a various biological effects, including anti-ulcer activity[22,23]. The health benefits of flavonoids are linked to their antioxidant activities with several studies demonstrating the ability of these compounds to mop up reactive oxygen species[24]. Flavonoids also possess membrane stabilizing properties[25] and some of the known flavonoids have been shown to increase gastric mucosal prostaglandin contents. Apart from the free radical scavenging ability of flavonoids, their antioxidant property may be due to its ability to chelate transition metal ions, inhibit oxidant enzymes, diminish acid secretions and inhibit the production of pepsinogen[26].

Saponins are a form of glycosides which derive their name from their soap-like effects which are due to their surfactant properties. Gastroprotective effects of saponins have been reported in various literature[27]. Tannins are known to possess styptic properties, due to their ability to react with the proteins of the tissue layers with which they come into contact. Tannins are said to ‘tan’ the outermost

layer of the mucosa rendering it less permeable and more resistant to injury or irritation. Application of tannins to the mucosa at a low concentration leads to precipitation of micro-proteins at ulcer sites forming a protective layer that makes it less susceptible to biological and chemical irritation[11].

The extracts of *V. doniana* may have exerted its antiulcerogenic property by one or more of the various mechanisms involving these active biological compounds mentioned above.

Considering the results obtained in this study, there is preliminary evidence that crude leaf extracts of *V. doniana* possess antiulcerogenic properties against ethanol induced gastric ulcer in rats.

Conflict of interest statement

We declare that we have no conflict of interest.

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