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Full Length Research Article

GASTROPROTECTIVE EFFECT OF AQUEOUS STEM BARK EXTRACT OF *ANOGEISSUS LEIOCARPUS* AGAINST ETHANOL-INDUCED GASTRIC ULCER IN RATS

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ABSTRACT
The Gastroprotective effect of *Anogeissus leiocarpus* aqueous stem bark extract against ethanol induced gastric ulceration in albino rats was investigated. The objective of the present investigation was to assess, in an animal model, the gastroprotective effects of aqueous stem bark extract of *Anogeissus leiocarpus* and to screen for possible protective effects of the extract as ulcer remedy. Fifty male and thirty six female albino rats (weighing 160-250g) were used in this study. Phytochemical studies revealed the presence of flavonoids, alkaloids, saponins, anthraquinones, tannins, cardiac glycosides, steroids and triterpenes. The median lethal dose (LD_{50}) studies of the aqueous stem bark of *A. leiocarpus* were found to be above 5000mg/kg body weight orally. Pre-treatment by oral administration of aqueous stem bark extract of *A. leiocarpus* at doses of 100, 200 and 400mg/kg b.w for 14 days, dose dependently significantly decreased the mean ulcer score, ulcer index, percentage ulceration and preventive index ($p<0.05$) induced by 70% ethanol. The standard drug (cimetidine 100mg/kg) also decreased the ulcer parameters. The severity of the reaction to ethanol on gastric mucosa and cytoprotection by aqueous *A. leiocarpus* were apparent by histological assessment of the gastric mucosa. In conclusion these findings shown that aqueous stem bark extract of *A. leiocarpus* possess a dose-dependent gastroprotection of the gastric mucosa ulcer parameters and cytoprotectection evident by histopathology. These justify the ethno medicinal use of the plant in the treatment and management of gastric ulcer.

Keywords: *Ethanol, Ulcer, Anogeissus leiocarpus, Cytoprotection*.

INTRODUCTION

Gastric ulcer is one of the major gastrointestinal disorders, which occurs due to an imbalance between the offensive (gastric acid secretion) and defensive (gastric mucosal integrity) factors (Laine *et al.,* 2008 and Shaker *et al.,* 2010). The incidence of peptic ulcer is increased due to stress, smoking, alcohol, Helicobacter pylori and ingestion of non-steroidal anti-inflammatory drugs (NSAID) (Vonkeman *et al.,* 2007; Ineu *et al.,* 2008; Sowndhararajan *et al.,* 2013).

Ethanol is also known as a cause of gastric damage by altering protective factors, including decreasing mucus production and blood circulation within the mucosa (Boligon *et al.,* 2014) In addition, the gastric damage caused by ethanol may be due to the generation of reactive species, decreased cell proliferation, and an exacerbated inflammatory response (Amaral *et al.,* 2013).

Several orthodox pharmaceutical drugs such as histamine H_2-receptor antagonists, antacids, anticholinergic drugs and proton-pump inhibitors have been used in the management of peptic ulcers, but they bring about many adverse effects (Mahdy *et al.,* 2018). Presently, there has been growing interest in alternative therapies especially from plant sources due to their perceived lower side effects, ease of accessibility and affordability (Rates, 2001, Bassi *et al.,* 2014 and Strand *et al.,* 2017).

Decoction from the plant has been used in folk medicine for the treatment of ulcers (Oluronti *et al*., 2012). There is need to validate these findings as a way to boost acceptability and hence usage of anti-ulcer therapy from stem bark of *Anogeissus leiocarpus*.

Anogeissus leiocarpus DC.Guill. & Perr. is a Combretaceae commonly called 'axlewood', locally referred to in Nigeria as '"Ayin", "Orin-odan" in Yoruba, "Marke" in Hausa and "Atara" in Igbo'. It is an evergreen tall tree found in savannah region of Tropical Africa, especially west and east Africa through tropical Southeast Asia (Steentoft, 1988; Odugbemi and Akinsulire, 2008). It is typically found growing at altitudes of 450 to 1900 m, and do grow on a range of soil types including compact clay soils (Vertisols) (Moctar and Sidi, 2007).

A. leiocarpus was reported to have high antimicrobial activities in many chemotherapeutic applications, hence its continued use in the treatment of bacterial infections (Mann *et al.,* 2009; Mann *et al.,* 2010; Mann, 2012). Antibacterial activity of alcohol extracts of leaf, stem bark and root bark of *Anogeissus leiocarpus* showed higher activity against Staphylococcus aureus than other test organisms. In vitro investigation of extracts of *A. leiocarpus* for antifungal activities against *Aspergillus niger, Penicillium species, Microsporum audouinii* and *Trichophyton rubrum* using radial growth technique displayed depression on rats (Mann *et al.,* 2008a; Mann *et al.,* 2008b).

The objective of the present investigation was to assess, in an animal model, the gastroprotective effects of aqueous stem bark extract of *Anogeissus leiocarpus* and to screen for possible protective effects of the extract as ulcer remedy. Gastroprotective effects were assessed using ulcer index and calculated protective index as well as histopathological examination. Ethanol-induced gastric ulcer in rats was carried out as a model for mimicking the Gastric ulcer disease in humans

MATERIALS AND METHODS

Experimental animals

Animals used in this study were purchased from the animal house of the Faculty of Pharmaceutical Science, Ahmadu Bello

University, Zaria. Albino rats of both sexes weighing 160-250g were housed in environmentally controlled room (25 ± 2ºC, 12 h light/dark cycles) for three weeks to acclimatize which they were divided randomly into five groups of six animals each coded to prevent observer bias. The animals were fed with standard feed (Vital feeds LTD Kano) and water ad *libitum*.

Preparation of plant materials

The plant was obtained from Zaria city in March 2017. It was identified and authenticated in the herbarium unit of the Department of Botany, Faculty of life sciences, Ahmadu Bello University, Zaria, and Voucher number 900389 was deposited.

Stem bark of *Anogeissus leiocarpus* was thoroughly washed and dried under the shade for 7 days. The stem bark was pulverized using pestle and mortar to fine powder (250g), placed in a mechanical shaker and exhaustively macerated in cold distilled water for 24 h. The mixture was allowed to settle and then filtered using a filter paper (Whatmann No 1). The filtrate was transferred into a petri-dish and concentrated in water bath at 40°C and was subsequently preserved as the extract. The extract obtained was weighed, and the percentage yield was calculated in term of air dried weight of the plant material as shown below:

$$Percentage\ yield = \frac{Weight\ of\ extract\ obtained}{Weight\ of\ initial\ sample} \times \frac{100}{1}$$

Median Lethal Dose (LD_{50}) Determination in Rats.

LD_{50} determination was conducted using Lorke's method (1983). This method was carried out in two phases. In the first (initial) phase, 3 rats per group of different weights were treated with the extracts at a dose of 10 mg/kg, 100 mg/kg and 1000 mg/kg body weight orally and were observed for signs of toxicity (weakness, drowsiness, lethargy) and death for 24 h.

In the second phase, 4 rats of different weights were administered doses of the extract at 1200mg/kg, 1600 mg/kg, 2900 mg/kg, and 5000 mg/kg body weight respectively based on the result of phase 1(i. e initial phase). The LD_{50} value was determined by calculating the geometric mean of the highest non-lethal dose (0/1) and lowest lethal dose (1/1) as shown in the formula below:-

$$LD_{50} = \sqrt{(Highest\ non\ lethal\ dose) \times (lowest\ lethal\ dose)}$$

Phytochemical analysis of the aqueous stem bark extract

Phytochemical analysis of the aqueous stem bark extract was carried out carried out using simple chemical tests to detect the presence of secondary plant constituents such as alkaloids (Ciulie, 1984), tannins (Ciulie, 1984), flavonoids (Ciulie, 1984), saponins (Brian and Turner, 1975), triterpenes and steroids (Rojjas *et al.,* 2006), cardiac glycoside (Evans, 2002), anthraquinones (Rahman, 2010).

The experimental protocol and ethanol-induced gastric lesions method

Group A–Normal control: Rats received only distilled water (0.5mL/100g body weight) (NC).

Group B–-Ulcer control treated with 70% ethanol: Rats received only 70% ethanol 1hr before sacrifice (0.5mL/100g body weight) (UC).

Group C–100mg/kg of the extract+ ethanol: Rat received 70% ethanol (0.5mL/100g body weight), 2 weeks after daily administration of aqueous stem bark extract of *A. leiocarpus* (100mg/kg body weight) (Al-100+ethanol).

Group D–200mg/kg of the extract+ ethanol: Rats received 70% ethanol (0.5mL/100gbodyweight), 2 weeks after daily administration of aqueous stem bark extract of *A. leiocarpus* (200mg/kg body weight) (Al-200+ethanol).

Group E –400mg/kg of the extract+ ethanol: Rats received 70% ethanol (0.5mL/100g body weight), 2 weeks after daily administration of aqueous stem bark extract of *A. leiocarpus* (400mg/kg body weight) (Al-400+ethanol).

Group F–cimetidine+ ethanol: Rats received 70% ethanol (0.5mL/100g body weight), 2 weeks after daily administration of cimetidine (100mg/kg body weight) (Cimetidine+ethanol).

Total number of animals in a group= 5

Gastric ulcer inducing dose of ethanol

To induce ulcers with ethanol, animals were fasted for 24–36 hours following which absolute ethanol was administered at a dose of 1mL/200g body weight to each animal and after 1hr the animals were sacrificed. It is recommended that for every study, a preliminary assessment be done to determine the effective dose required for optimum induction of ulcers (Hollander *et al.,* 1985). An hour after the ethanol administration the animals were sacrificed by cervical dislocation followed by an abdominal incision. The stomach was removed and afterwards incised along the greater curvature. It was washed gently in running tap water and gastric mucosa spread on a filter paper for gastric lesions assessment. A 2x hand lens was used to locate the ulcers. Stomach ulceration was expressed in terms of: ulcer score, ulcer index, preventive index and % of ulceration.

Determination of Ulcer parameters

Ulcer score was assessed using the method of Takagi and Okabe (1968). Severity of mucosal damage was assessed on a score grade as follows;

0=no lesion,
1=mucosal oedema and petechiae,
2= one to five small lesions (1-2mm),
3=more than five small lesions or one intermediate lesion (3-4mm),
4=two to more intermediate lesions or one gross lesion (>4mm),
5=perforated ulcers.

***Mean Ulcer index*:** it's calculated by dividing total number of ulcers in animals in a group by number of animals in that group (Robert *et al.,* 1968).

Preventive index:

The preventive effect on the severity of ulceration was calculated according to the method of Hano *et al.,* (1976).

$$\text{Preventive index} = \frac{U.I\ control - U.I\ Treated}{U.I\ Control} \times \frac{100}{1}$$

Percentage of ulceration:

$$\text{The ulcer incidence (\%)} = \frac{Number\ of\ animals\ with\ ulcer}{Number\ of\ animals\ in\ a\ group} \times \frac{100}{1}$$

Histopathology studies of the stomach tissues

The stomach tissue samples were fixed in 10% buffered formalin overnight and then processed in an automated tissue processor. Stomach tissues were embedded in paraffin, sectioned by a microscope and stained with haematoxylin and eosin stain. Each section was examined by light microscope with magnification of ×400

Statistical Analysis

The results were expressed as mean ±standard error of mean

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(SEM). Statistical comparisons were performed by one-way analysis of variance (ANOVA), followed by Dunnets post-hoc test. The data was analyzed by using Statistical Package for the Social Sciences (SPSS, version20.0). A P-value less than 0.05 were considered to be significantly different.

RESULTS

The results of quantitative analysis of phytochemical constituents of aqueous stem bark extract of *Anogeissus leiocarpus* shows the presence of flavonoids, alkaloids, saponins, Anthraquinones, tannins cardiac glycosides, steroids and triterpenes (Table 1)

Oral administration of aqueous stem bark extract of *Anogeissus leiocarpus* for 24h showed no symptoms of toxicity and none of the rats died up to a dose of 5000 mg/kg b.w

Table 1: Qualitative screening of phytochemical constituents of crude aqueous stem bark extract of *Anogeissus leiocarpus*

Constituent	Remark
Flavonoids	+
Alkaloids	+
Saponins	+
Anthraquinones	+
Tannins	+
Cardiac glycosides	+
Steroids and triterpenes	+

Key: +, Present

Table 2: Effect of aqueous stem bark extract of *Anogeissus leiocarpus* on gastric mucosal membrane of ethanol-induced ulceration in albino rats.

Group	Mean ulcer index	% Ulceration	Preventive index
Normal Control	0	0	0
Ulcer Control	5.4±0.40^{a}	100^{a}	0
Al-100mg/kg + ethanol	2.8±0.37^{b}	100^{a}	48
Al-200mg/kg + ethanol	2.2±0.68^{c}	80^{b}	59
Al-400mg/kg + ethanol	1.4±0.87^{d}	40^{c}	74
Cimetidine-100mg/kg+ ethanol	2.6±0.68^{e}	100^{a}	52

Data represent the Mean± SEM. (n=5)

Different superscripts along the column are significantly different at $p<0.05$

Mean ulcer score expressed as mean ulcer index expresses the degree of ulceration on mucosal membrane. It was determined by naked eye using a hand lens as described in 2.5.1. The mean ulcer score in all the groups showed a significant dose-dependent decrease after ulcer induction by 70% ethanol (1ml/200gm b.w.) for 1hour when compared with the ulcer control. (Table 2)

The preventive index was calculated from ulcer index of control and treated group. Pretreatment with aqueous *Anogeissus leiocarpus* stem bark extract at various concentrations 100, 200 & 400 mg/kg b. w, produced a significant dose-dependent protective effect against gastric ulcers induced by 70% EtOH (1ml/200g b. w.) (Table 2)

Pretreatment with aqueous *Anogeissus leiocarpus* stem bark extract at a dose 100mg/kg b. w for 14 days, has a preventive index of 48%, 200mg/kg b.w of 59% and 400mg/kg b.w. of 74%. Cimetidine (standard) at a dose of 100mg/kg b.w has a preventive index of 52% which falls in between the preventive indices of 200 and 400 mg/kg b. w of the aqueous *Anogeissus leiocarpus* stem bark extract.

Histological evaluations on the effect of crude aqueous stem bark extract of *Anogeissus leiocarpus* and cimetidine on ethanol induced gastric lesions in rats (hematoxylin and eosin, 400×). Photomicrograph of gastric mucosa from normal control rats demonstrates an intact surface mucosal cell and gastric pits (Plate I). The ulcer control group had intense ulcerated gastric mucosal epithelial cells, necrotic tissue and heavy infiltration (Plate II). There were differences in the histopathology of the stomach in the different groups, where 100 mg/kg, 200 mg/kg and 400 mg/kg of the crude aqueous stem bark extract of *Anogeissus leiocarpus* were administered per kg body weight of the animals for 14 days with administration of 70% ethanol (1ml/200g b. w.) as shown in the photomicrograph (Plate III-IV) as compared with the group treated with standard drug Cimetidine (Plate VI). The section of gastric mucosa from rat pre-treated with stem bark aqueous extract of Anogeissus *leiocarpus* at 100 and 200mg/kg b. w. had slightly eroded mucosal epithelial cells, less infilteration and haemorrhage, as shown in Plates III and V respectively. In the 400mg/kg b.w. stem bark aqueous extract of *Anogeissus leiocarpus* pre-treated rat, there is no observable haemorrhagic necrosis of gastric mucosa and showed protection against the histopathological changes observed in ethanol treated group with an intact gastric pits, maintenance of mucosa even after exposure of ethanol (Plate V). Cimetidine (100mg/kg b.w.) pre-treated group demonstrates slight ulceration, less hemorrhagic necrosis, infiltration in the gastric mucosa of rat (Plate VI)

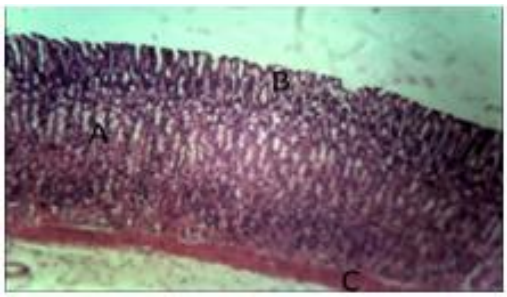

Plate I: Normal control group
A= Gastric pits, B= Gastric glands, C= Muscularis mucosae

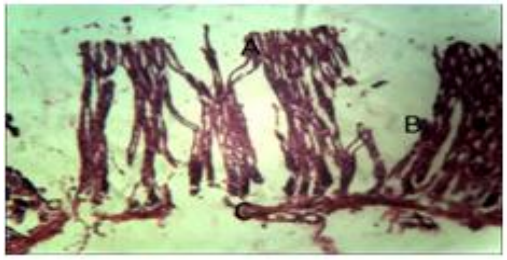

Plate II: Ulcer control group B
A= Gastric glands, B= Gastric pits, C= Muscularis mucosae

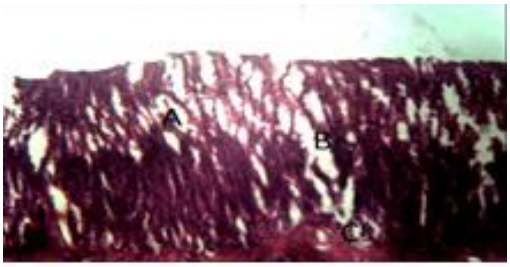

Plate III: Al-100mg/kg+ ethanol group C
A=Gastric pit, B= Gastric gland, C= Muscularis mucosae

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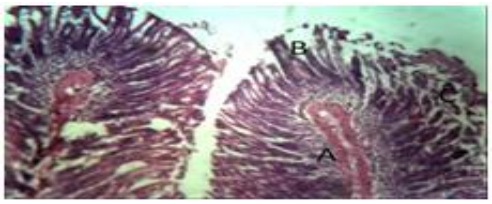


Plate IV: Al-200mg/kg +ethanol group D
A=Muscularis mucosae, B=Gastric pits, C=Gastric glands

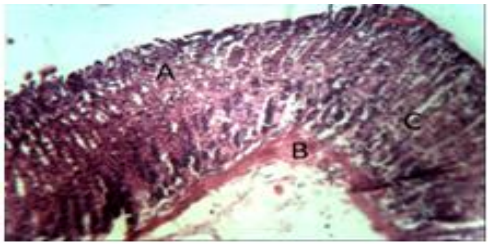


Plate V: Al-400mg/kg+ ethanol group E
A=, Gastric pits B=Muscularis mucosa, C=Gastric gland

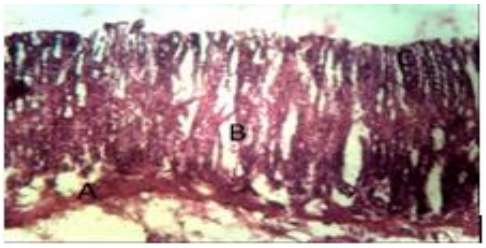
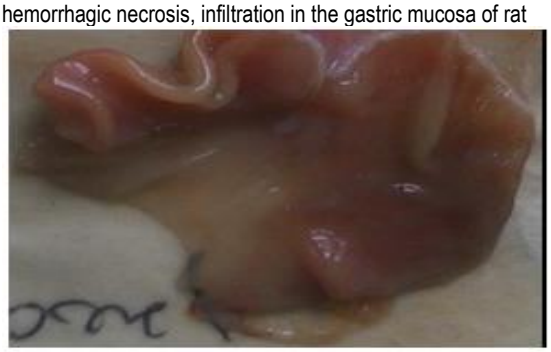
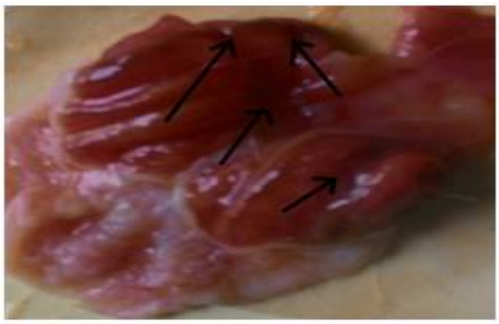


Plate VI: Cimetidine-100mg/kg + ethanol group F
A=Muscularis mucosae, B=Gastric gland, C=Gastric pit

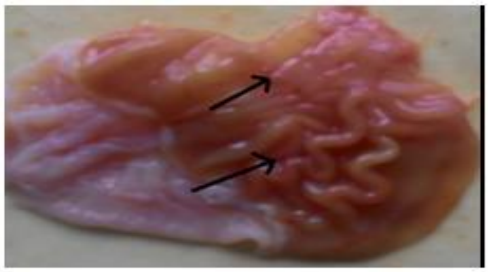
Figure 2: Histopathological findings of stomach sections from fasting rats H&E stain ×400: Plate 1: Photomicrograph of normal gastric mucosa of rat from normal rats demonstrates an intact surface mucosal cell and\gastric pits. Plate II: The ulcer control group had intense ulcerated gastric mucosal epithelial cells, necrotic tissue and heavy infiltration. Plate III: The Al-100mg/kg extract+ ethanol group showed slightly eroded mucosal epithelial cells, less infilteration and haemorrhage. Plate VI: The Al-200mg/kg extract+ ethanol group showed slightly eroded mucosal epithelial cells, less infilteration and haemorrhage. Plate V: Al-400mg/kg+ ethanol group showed no observable haemorrhagic necrosis of gastric mucosa, intact gastric pits and maintenance of mucosa even after exposure of ethanol. Plate IV: Cimetidine-100mg/kg + ethanol group showed slight ulceration, less hemorrhagic necrosis, infiltration in the gastric mucosa of rat



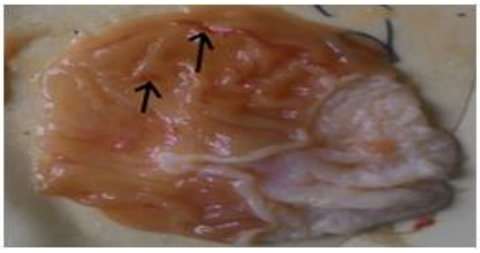
Normal control group A



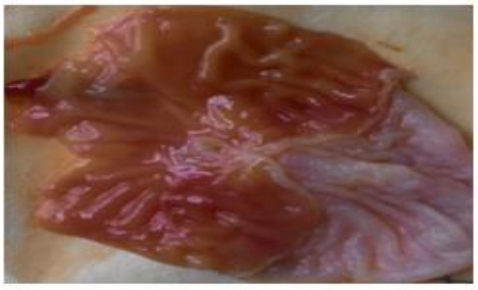
Ulcer control group B



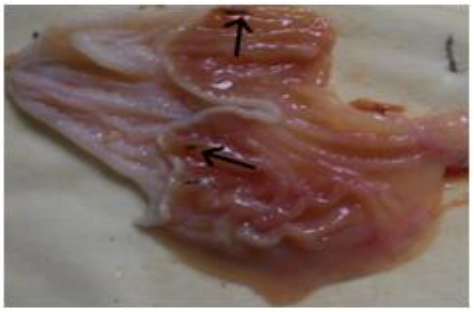
Al-100mg/kg+ ethanol group C



Al-200mg/kg +ethanol group D



Al-400mg/kg+ ethanol group E



Cimetidine-100mg/kg + ethanol group F

Figure 1: Effect of pre-treatment with aqueous stem bark extract of *Anogeissus leiocarpus* for 14 days on stomach lesions induced by 70% ethanol in rats. A: normal stomach mucosa. B: untreated group receiving only ethanol. C: Al-100mg/kg + ethanol group. D: Al-200mg/kg + ethanol group. E: Al-400mg/kg + ethanol group. F: Cimetidine-100mg/kg + ethanol group.

DISCUSSION

The increased frequency of occurrence of gastric ulcers in humans, severe side effects and cost of some available synthetic drugs, arises the use of natural products an important alternative treatment (Bassi, *et al.,* 2014 and Strand *et al.,* 2017). In this sense aqueous stem bark of *Anogeissus leiocarpus* have proven to be advantageous in the treatment of various diseases in lab animals and patients (Shuaibu *et al.,* 2005, Atawodi *et al.,* 2011, Victor and Grace, 2013, Timothy *et al.,* 2015).

Toxicity studies LD_{50} of aqueous stem bark extract of *Anogeissus leiocarpus* in rats indicated a no lethal effect up to a dose of 5000mg/kg body weight for 24 hours suggesting that the extract has a wide margin of safety.

Plants are known to contain a variety of secondary metabolites. These secondary metabolites or bioactive compounds produce definite physiological actions on the human system. Many of these phytochemicals have been discovered and even isolated from a variety of medicinal plants. Unfortunately, however, not many of them have been exploited for clinical use (Ekwueme *et al.,* 2015). Phytochemical analysis of plants is predicated by the need for drug alternatives of plant origin, made imperative by the high cost of synthetic drugs as in the case of anti-ulcers. These secondary plant metabolites extractable by various solvents exhibit varied biochemical and pharmacological actions in animals when ingested (Nwogu *et al.,* 2008). The aqueous stem bark extract of *Anogeissus leiocarpus* contains alkaloids, flavonoids saponins, tannin, steroid and triterpenes, anthraquinones and cardiac glycosides (Table 1).

The presence of flavonoids in the aqueous stem bark extract of *Anogeissus leiocarpus* could account for its use as an antinflammatory agent (Ekwueme *et al*., 2015). It also means that the plant could be used to prevent damage caused by free radicals in the body (Dweck and Mitchell, 2002). Oxidative stress induced by ethanol was suppressed in the treatment group which proves the radical scavenging activity of the extract. Flavonoids exhibit dramatic effects on immune and inflammatory cells; these can be either immunosuppressant or immune stimulatory (Huang *et al.,* 2010). This was evident from the histopathological assessment of the gastric tissues. Tannins are known to possess immune-stimulating activities. This suggests the possible potential of *Anogeissus leiocarpus* in the treatment of dysentery, diarrhea, bacterial infection and in wound healing.

In the present study, oral administration of 70% ethanol to rats produced gastric mucosal lesions which correspond to score grade 3 (more than five small lesions or one intermediate lesion 3-4mm) by the method of Takagi and Okabe (1968) of severity of mucosal damage assessment. Macroscopic examination with determination of mean ulcer index, preventive index and percentage ulceration was carried out. Ulcers caused by ethanol may possibly arise from direct damage of gastric mucosal cells, abnormal elevation of reactive species which correspond to one of the main aggressive mechanism of ethanol mediated ulceration (Amaral *et al.,* 2013).

The histological findings of the stomach tissue in accordance with previous studies of ulcer control group showed typical histological damage 1 h after ethanol administration. The damage was characterized by intense ulcerated gastric mucosal epithelial cells, necrotic tissue and heavy infiltration (Gomez-Guzman *et al.,* 2018). The aggregation of neutrophils plays a fundamental role in the process of injury and inflammation in the gastric mucosa due to their release of tissue-disruptive substances like proteases, leukotrienes B4 (LTB4), and reactive oxygen species Via NADPH oxidase, neutrophils release superoxide anions, and these in turn are metabolized into the hydroxyl radical. The latter can mediate lipid peroxidation of polyunsaturated fatty acids and cause damage to cell membranes, leading to an alteration in the structural integrity and biochemical function of membranes (Naito *et al.,* 1995 and Kobayashi *et al.,* 2001). Pre-treatment with 100 and 200 mg/kg b.w of aqueous stem bark extract of *Anogeissus leiocarpus* showed slightly erosion, less infilteration and hemorrhage to the gastric mucosa cells, intact gastric pits and surface mucosal cells. 400mg/kg b.w. of aqueous stem bark extract of *A. leiocarpus* conferred a high protection of the histological structures with no observable hemorrhagic necrosis of the gastric mucosa as well as intact gastric pits and muscularis mucosae. Pre-treatment with cimetidine was protective however there were some histological injuries in the gastric mucosa of the stomach. The result showed that the gastroprotection from the aqueous stem bark extract of *Anogeissus leiocarpus* used was dose dependent on the dosage as also showed from the gastric ulcer score result.

Oxygen derived radicals and agents with antioxidant properties have been implicated in the pathogenesis of ethanol induced ulcers (Boligon *et al.,* 2014). Alcohol rapidly penetrates the gastric mucosa apparently causing cell and plasma membrane damage leading to increased intracellular membrane permeability to sodium and water. The massive intracellular accumulation of calcium represents a major step in the pathogenesis of gastric mucosal injury (Raju *et al.,* 2009). Ethanol-induced gastric ulcer can arise as a result of direct damage to mucosal cells, development of free radicals and hyper oxidation of lipid (Terano *et al.,* 1989). But scavenging these free radicals can play a role in healing of these ulcers (Halliwell and Gutteridge, 1992). Oxidative stress has been showed to play a role in alcohol-induced gastric mucosal damage (Gomez-Guzman *et al.,* 2018 and Rufa'i *et al.,* 2021). The gastric protective effect of aqueous stem bark extract of *Anogeissus leiocarpus* is a result of its antioxidant effect (Rufa'i *et al.,* 2021).

Conclusion

The results from the study shown that aqueous stem bark extract of *Anogeissus leiocarpus* (100, 200, 400mg/kg), demonstrated a gastroprotective effect against ethanol-induced gastric ulceration in rats. *A. leiocarpus* possess a dose-dependent gastroprotection of the gastric mucosa ulcer parameters and cytoprotectection evident by histopathology. This is as a result of the antioxidant properties of the extract carried out in a study by the authors.

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