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Evaluation of the Antiulcerogenic Effects of N-Hexane Fraction of *Dialium guineense*, Willd Leaves on Indomethacin-Induced Ulcer on Adult Albino Wistar Rat

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Abstract

Developing countries are burdened by a low standard of living, resulting in stressful conditions that expose the citizens to many health challenges, like peptic ulcer disease (PUD). Prevalence of fake and substandard drugs with the attendant side effects has resulted in the interest and use of ethnomedicine. This study aimed to evaluate the curative antiulcer effects of the n-hexane fraction of *Dialium guineense*, Willd (Fabaceae) (DG) leaves on the NSAID-induced model of PUD in adult Wistar rats. *D. guineense*, leaves were subjected to extraction, fractionation, phytochemical analyses, and determination of lethal dose (LD_{50}). Adult Wistar rats (36), divided into 6 groups of 6 per group, were used. Group 1 was a normal control, while groups 2 - 6 were induced peptic ulcer with indomethacin (40 mg/kg) via oral gavage. Group 2 animals served as ulcer control, group 3 received the standard drug (omeprazole 20 mg/kg), groups 4 - 6 received 100, 200, and 400 mg/kg of n-hexane fraction, respectively. The animals were treated 14 days after the administration of indomethacin. On the 15th day, blood samples were taken, and the animals were sacrificed by cervical dislocation, and their stomach were isolated for ulcer index and histopathology. The antioxidant activity of the fraction was investigated by examining its effects on superoxide dismutase (SOD), malondialdehyde (MDA), and reduced glutathione (GSH). catalase (CAT) activity was also assessed. Free acidity, total acidity, and pH values were estimated. Phytochemical analyses showed the presence of terpenoids, alkaloids, and steroids. The results of the LD_{50} showed neither deaths nor signs of acute toxicity up to 5000 mg/kg dose. There was little or no ulceration in the n-hexane fraction treated groups. Antioxidant studies showed significantly increased values of SOD and significantly decreased values of MDA, supporting positive antioxidant activity; CAT and GSH values were not affected. Free acidity and total acidity were significantly decreased by 100 and 200 mg/kg of N-hexane fraction. Histopathology showed normal stomach histoarchitecture of the treatment group which compared favorably to the standard group,

Keywords: *Dialium guineense,* n-hexane fraction. indomethacin, ulcer, rats.

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Introduction

Most developing countries, like Nigeria, are plagued by poor leadership, which is evident in the living conditions of the citizens. Citizens suffer health challenges strongly linked to stress and bad environmental conditions, and one such disease is peptic ulcer disease. Peptic ulcer disease (PUD) is the erosion or loss of the mucosal lining of the epithelial surface of the stomach or duodenum, sometimes extending to the muscularis mucosa. PUD is caused by the action of acid pepsinogen complex on the mucosal layer, leading to gaps in the continuity of the mucosal layer. PUD is reported to be the most common disorder of the gastrointestinal tract, resulting mainly as a result of an imbalance between the gastric defensive and aggressive factors Mukhet *et al*, 2019). Defensive factors against peptic ulcer are adequate blood flow, secretion of prostaglandins, mucin, bicarbonate, mucus bicarbonate layer, cellular regeneration, mucosal barrier, surface mucus secretion, and secretion of nitric oxide (Bongu and Vijayakumar, 2012. Thabrew and Arawwawala, 2016; Mehta, 2016; Thomas *et al*., 2017). Aggressive factors were reported to be reactive oxygen species, increased secretion of hydrochloric acid and pepsin, inadequate dietary habits, free oxygen radicals, consumption of NSAIDs, alcohol, stress, and anxiety Cuevas *et al*., 2011).
PUD is treated using drugs that are antacids, proton pump inhibitors, antihistamines, anticholinergics, etc. These drugs have side effects and other adverse reactions, but the problem with developing countries is deep-rooted corruption in the system. Fake drugs are rife, and underdosed drugs also create another problem for drug manufacturers and importers. Some agencies check the standardization of these drugs, but the problem of underdosing and fake drugs is widespread, affecting nooks and crannies in urban, suburban, and rural areas.

Consequent on this trend, people are increasingly resorting to ethnomedicine, which is not only accessible and affordable, but has no fear of fakeness. N-hexane, a nonpolar solvent, is commonly used to extract nonpolar phytochemicals like lipids, fats, oils, waxes, and certain terpenoids. It's also used in the defatting process to remove oils and waxes before extracting other compounds. Additionally, n-hexane can extract some alkaloids, flavonoids, saponins and phenols, though polar solvents extract these phytochemicals more efficiently. Some studies have shown the presence of other compounds like glycosides and tannins in n-hexane extracts, but these are less frequently reported as being efficiently extracted with this solvent.

The choice of solvent is crucial for successful phytochemical extraction. N-hexane is best suited for nonpolar compounds. For polar compounds, other solvents like methanol or ethanol are generally preferred.

Phytochemicals are naturally occurring bioactive compounds found in plants, often giving them their color, aroma, and flavour. They are not considered essential nutrients but are believed to contribute to human health by offering protection against various diseases. Phytochemicals act as antioxidants, protect against cellular damage, and may reduce the risk of chronic diseases, including cancer and heart disease. Siddiqui and Moid (2022) posited that nearly 80% of the world's population makes use of natural medicine in primary health care.

Many plants show anti-ulcer potential due to the presence of phytochemicals that have antioxidant and antimicrobial activities. They can improve mucosal defense, reduce acid production, and combat *Helicobacter pylori* (Agu *et al*., 2023), Phytochemicals that have putative antiulcerative properties are alkaloids, tannins, flavonoids, terpenoids, glycosides, carotenoids, and saponins (Ghosh *et al,* 2016., Agu *et al*., 2023). The plant *Dialium guineense* Wild (family Fabaceae, subfamily Caesalpinioideae), grows in dense savannah forests, shadowy canyons and gallery forests. It is native to Nigeria, Benin Republic, Burkina Faso and other West African countries. It is called "icheku" (Ibo, Eastern Nigeria), "awin" (Yoruba, Western Nigeria), "tsayirarkurm" (Hausa, Northern Nigeria), velvet termarind or black tamarind (English) and tamarinier noir (French). The leaves and stem bark are used as folklore remedies for the treatment of infections and other ailments such as diarrhea, severe cough, bronchitis, wound, stomach ache, malaria fever, jaundice, peptic ulcer disease (PUD), hemorrhoids, and prevention of cancer (Bero *et al*., 2009., Gideon *et al* 2013). *D. guineense* leaves, also known as velvet tamarind leaves, possess significant nutritional potential. They are a source of various vitamins and minerals, including vitamins C and E, and are also rich in phytochemicals like alkaloids, tannins, phenols, and flavonoids. These components contribute to the plant's antioxidant and antimicrobial properties, potentially offering health benefits (Ginovyan *et al.,* 2017; Cheesman *et al.,* 2018). Terpenoids may also possess medicinal effects such as anti-mutagenic, anti-ulcer, antimicrobial, anti-malarial, and anti-cancer (Saxena *et al.,* 2013). They are a source of iron, magnesium, and copper. This study aimed to evaluate the curative antiulcer effects of the n-hexane fraction of *Dialium guineense* leaves on an indomethacin-induced model of PUD in adult Wistar rats.

Materials and Methods

Plant material

The plant leaves were collected from Agu Ibagwa Forest in Nsukka LGA and identified by Isaac Ossai, a taxonomist from the Biodiversity and Conservation Programme

(BDCP), Nsukka, 36 Aku Road, Nsukka. A specimen voucher with number BDCP/C24/002 was issued.

Extraction and fractionation of the plant material

The extraction procedure was carried out according to Girma *et al* (2015) with slight modifications. The leaves were shade-dried and pulverized. The pulverized leaves were macerated in 1000 ml of methanol for 48 h, and filtered with Whatman filter paper. The filtrate was concentrated using a rotary evaporator. The concentrated crude methanol extract was successively eluted with n-hexane, dichloromethane, ethyl acetate, and water using column chromatography technique. The n-hexane fraction was used in this study.

Qualitative and quantitative phytochemical analyses

The methods of Harbone (1978) and Trease and Evans (1989) were used to qualitatively and quantitatively determine alkaloids, flavonoids, tannins, total phenolics, glycosides, reducing sugars, steroids, and terpenoids.

Animals

Adult male Wistar rats (24) weighing 150 ± 20 g, and mice (20-25 g) were purchased from the Animal House of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka. The rats were housed in cages in the Animal House of Anatomy Department, University of Nigeria, Enugu Campus under standard environmental condition. The animals were fed with laboratory chow (Chikun feeds PLC) and drinking water *ad libitum* for a period of two weeks in order for them to acclimatize to the new laboratory environment prior the study. Natural light and dark cycles maintained, and temperature was kept between 27℃ and 30℃.

Acute toxicity (LD_{50}) study

Mice were used for the acute toxicity determination according to Lorke's method (1983). Observations were made for any sign of toxicity and mortality for twenty-four hours; observations were made for any possible death(s).

Experimental Design

Thirty six albino Wister rats were used in the anti-ulcer study. They were they were randomly divided into six groups (n=6) as follows:
Group 1 Normal control
Group 2 Induction (indomethacin 40 mg/kg bw) + No treatment
Group 3 Indomethacin + Standard (omeprazole 20 mg/kg)
Group 4 Indomethacine + N-hexane Fraction of *D. guineenses* 100 mg/kg
Group 5 Indomethacin + N-hexane Fraction of *D. guineenses* 200 mg/kg
Group 6 Indomethacin + N-hexane Fraction of *D. guineenses* 400 mg/kg

The rats were induced with indomethacin (40 mg/kg) and then treated with fractions and the standard drug for 14 days. On the 15th day, the animals were fasted for 8 hours before they were sacrificed by cervical dislocation, and their stomachs were opened along the greater curvature and scored for degrees of ulceration according to the method of Urushidani (1979). The stomachs were then fixed in 10 % phosphate-buffered formalin for the histopathological examinations.

Determination of ulcer index

The stomachs were opened along the greater curvature and rinsed with water to remove gastric contents and blood clots, and examined by a 10× magnifier lens to assess the formation of ulcers. The number of ulcers was counted. Dashputre and Naikwade (2011) method of scoring of ulcer was made as follows:

Normal colored stomach (0), Red coloration (0.5), Spot ulcer (1), Hemorrhagic streak (1.5), Deep ulcers (2), Perforation (3).

The total mucosal area and total ulcerated area were measured. The ulcer index was then calculated using the following equation (Melese *et al,* 2011):

Ulcer index=10/A

where A is the total mucosal area/ulcerated area. Percentage inhibition of ulceration was calculated thus:

%inhibition of ulceration= $UIc - UIt/UIc\ x\ 100$

UIc=ulcer index control group, UIt=ulcer index test group

In vivo antioxidant studies

The *in vivo* antioxidants that were determined were superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), and the lipid peroxidation marker malondialdehyde (MDA). Catalase activity was assayed using the method of Aebi (1983). The product of lipid peroxidation (MDA) was assayed using the method of Wallin *et al.* (1993). The superoxide dismutase activities were determined using the method of Xin *et al.* (1991).

Determination of pH

Gastric acid was obtained by ligation of the pylorus and carefully aspirating the stomach gastric juice with a syringe. An aliquot of 1 ml of gastric juice was diluted with 1 ml of distilled water, and the pH of the solution was measured using a pH meter (Adwa AD8000) (Abebaw *et al,* 2017).

Determination of total acidity and free acidity

This is based on simple acid-base titration. Gastric juice (1mL) was pipetted into a 100 mL conical flask and diluted with 9 mL distilled water. Two or three drops of Topfer's reagent then added and titrated with 0.01 N sodium hydroxide until all traces of red color disappeared and the colour of the solution was yellowish-orange. The volume of alkali added was noted. This volume corresponds to free acidity. Two or three drops of phenolphthalein were then added, and the titration was continued until a definite red ting appeared; the volume of alkali added was noted. The volume corresponds to total acidity. Acidity was expressed in terms of mEq/L (Vivek *et al*, 2014).

Histopathological Examinations

The histopathological examinations of the stomach of the albino Wistar rats were done using the method of Drury *et al*. (1967). It involved fixing in formalin and washing of the tissues. Dehydration was achieved using alcohol, and the embedding was done in paraffin. They were subsequently cleared, infiltrated with paraffin wax, sectioned, mounted on the slides, and stained with eosin and haematoxylin. The slides prepared were mounted on a photomicroscope one after the other and viewed at x 400 magnification of the microscope.

Statistical Analysis

Data were presented as mean ± SD. One-way analysis of variance (ANOVA) was used to analyze the experimental data. Duncan multiple test range was used to separate the means and differences were considered significant at $P < 0.05$ using statistical package for service solution (SPSS) version 23.

Results

Results of the qualitative and quantitative analyses

The result of quantitative phytochemical analyses indicate the presence of flavonoids, tannins and total phenolics alkaloids, steroids and terpenoids. Saponin and Glycosides were not detected. (Table 1)

Table 1: Qualitative and quantitative phytochemical results analyses

Phytochemicals	Bioavailability	Amount (mg/100g) mean±SD
Saponin	ND	00.00
Glycosides	ND	00.00
Reducing sugar	++	155.44±3.39
Alkaloids	++	304.03±45.48
Flavonoids	+	164.06±45.11
Tannins	+	21.07±4.85[a]
Total phenolics	+	6106.18±672.46
Steroids	++	13.97±646.45
Terpenoids	++	318.11±34.97

Acute toxicity (LD_{50}) test

There was no mortality up to 5000 mg/kg indicating that the LD_{50} of the extract is more than 5000 mg/kg

Effect of the fraction on indomethacin-induced ulcer

The stomachs of the fraction treated rats did not show any ulceration.as opposed to the untreated control. Thus, the ulcer index could not be calculated.

Effect of the fraction on I the Antioxidants and Lipid Peroxidation Marker

The fraction caused a significant ($P < 0.05$) increase in the level of SOD and. reduced glutathione (GSH) and Catalase (CAT) activities. The lipid peroxidation marker malondialdehyde (MDA) was significantly ($P < 0.05$) (Table 2).

Effect of the fraction on Free acidity, Total acidity and, PH

Treatment with the fraction did not significantly ($p > 0.05$) affect the pH however the free and total acidity were significantly decreased ($P < 0.05$) (Table 3)

Table 2: Effect of the fractions on *In vivo* Antioxidants and Lipid Peroxidation Marker

Groups	GSH (mg/dl)	MDA (mg/dl)	SOD (IU/L)	CAT IU/L
NC	0.88 ± 0.42^{ab}	0.74 ± 0.16^{a}	11.42 ± 0.07^{b}	1.05 ± 0.34^{a}
Indo	1.50 ± 0.30^{b}	2.05 ± 0.98^{b}	10.71 ± 0.44^{a}	2.69 ± 0.45^{b}
Omep	0.58 ± 0.11^{a}	0.77 ± 0.13^{a}	11.41 ± 0.06^{b}	0.92 ± 0.10^{a}
Fr 100	0.85 ± 0.08^{ab}	0.66 ± 0.11^{a}	11.45 ± 0.04^{b}	1.04 ± 0.23^{a}
Fr 200	1.18 ± 0.46^{ab}	0.78 ± 0.09^{a}	11.48 ± 0.01^{b}	0.92 ± 0.49^{a}
Fr 400	0.77 ± 0.05^{ab}	0.92 ± 0.07^{a}	11.48 ± 0.01^{b}	0.91 ± 0.26^{a}

NC- Normal control, Indo-Induction (indomethacin 40 mg/kg) + No treatment, Omep- (omeprazole 20 mg/kg), Fr 100- n-hexane fraction *(*100 mg/kg), Fr 200- n-hexane fraction (200 mg/kg), Fr 400-nN-hexane fraction (400 mg/kg). Superscripts that are different were considered significant ($P < 0.05$).

Table 3: Effect of the fraction on Free acidity, Total acidity and PH

Groups	Free acidity (µM/µl)	Total acidity (µM/µl	pH
NC	0.03 ± 0.01	0.05 ± 0.01	3.83 ± 0.44
Indo	$0.15\pm0.02^{b,c,d}$	0.24 ± 0.07^{cb}	$4.08\pm0.15^{a,b}$
Omep	$0.14\pm0.04^{a,b,c,d}$	0.22 ± 0.05^{cd}	3.47 ± 0.105^{a}
Fr100	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}	3.5 ± 0.40^{a}
Fr200	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}	3.4 ± 1.20^{a}
Fr400	$0.10\pm0.09^{a,b,c,d}$	$0.11\pm0.10^{a,b}$	$4.1\pm1.13^{a,b}$

NC- Normal control, Indo-Induction (indomethacin 40 mg/kg) + No treatment, Omep- (omeprazole 20 mg/kg), Fr 100- n-hexane fraction *(*100 mg/kg), Fr 200- n-hexane fraction (200 mg/kg), Fr 400-nN-hexane fraction (400 mg/kg). Superscripts that are different were considered significant ($P < 0.05$).

Histopathological studies

Photomicrograph the normal control rats showed normal stomach architecture with intact gastric mucosa (M), sub-mucosal (SM) and muscularis externa (ME) layer. Mucosa shows intact columnar epithelium (E), well-defined gastric pits (GP) and glands (GG). Submucosa contained connective tissue, while the muscularis externa displayed distinct inner circular and outer longitudinal muscle layer. No pathological changes such as inflammation or ulceration were (Figure 1). Administration of indomethacin resulted in shedding and ulceration in gastric epithelium and gastric pit. Sub-mucosal and muscularis external layers were intact. Submucosa had connective tissue, blood vessels, and nerve fibers, while the muscularis externa displayed distinct inner circular and outer longitudinal muscle layer (Figure 2)

Photomicrograph of the stomach treated with omeprazole (40 mg/kg) showed mild shedding and ulcers in gastric epithelium. Sub-mucosal and muscularis externa layers were intact. Submucosa contained connective tissue while the muscularis externa displayed distinct inner circular and outer longitudinal muscle layer (Figure 3). The stomachs of the rats treated with the fraction (100 mg/kg) showed normal stomach architecture with intact gastric mucosa, sub-mucosal and muscularis externa layers. Mucosa had intact columnar epithelium, well-defined gastric pits and gastric glands. Submucosa contained connective tissue, while the muscularis externa displayed distinct inner circular and outer longitudinal muscle layer. No

pathological changes such as inflammation or ulceration were observed (Figure 4).

Treatment with the fraction (200 mg/kg) resulted in normal or lesion regenerated area on surface columnar epithelium (thick black arrows). The submucosa contained connective tissue, while the muscularis externa displayed distinct inner circular and outer longitudinal muscle layer (Figure 5). Photomicrograph of the stomach treated with the fraction (400 mg/kg) showed normal stomach architecture with intact gastric mucosa, sub-mucosal, and muscularis externa layer. The mucosa had intact columnar epithelium, well-defined gastric pits, and glands (GG). Submucosa contained connective tissue, while the muscularis externa displayed a distinct inner circular and outer longitudinal muscle layer. No pathological changes, such as inflammation or ulceration were evident (Figure 6)

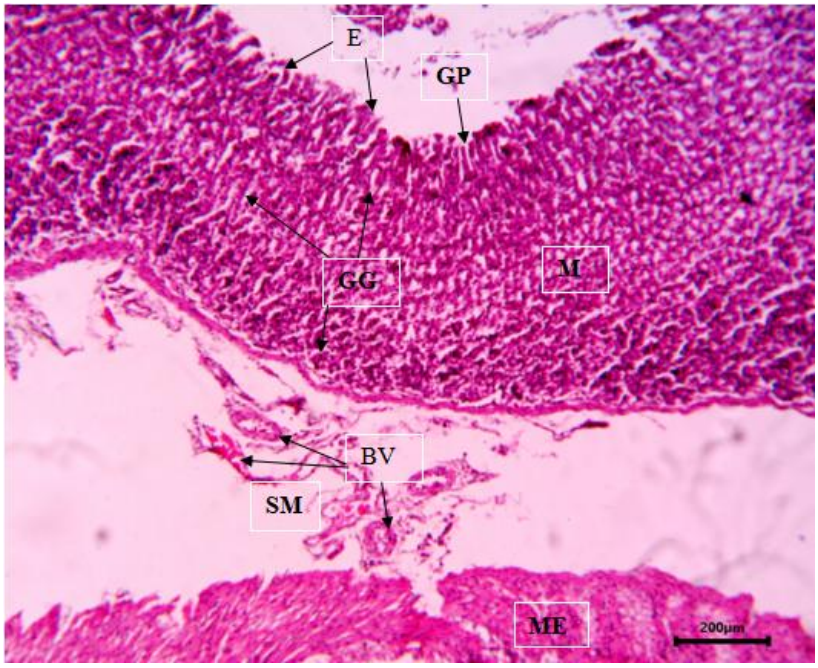

Figure 1: Photomicrograph of group 1 (Normal control) of the curative study H&E, x100

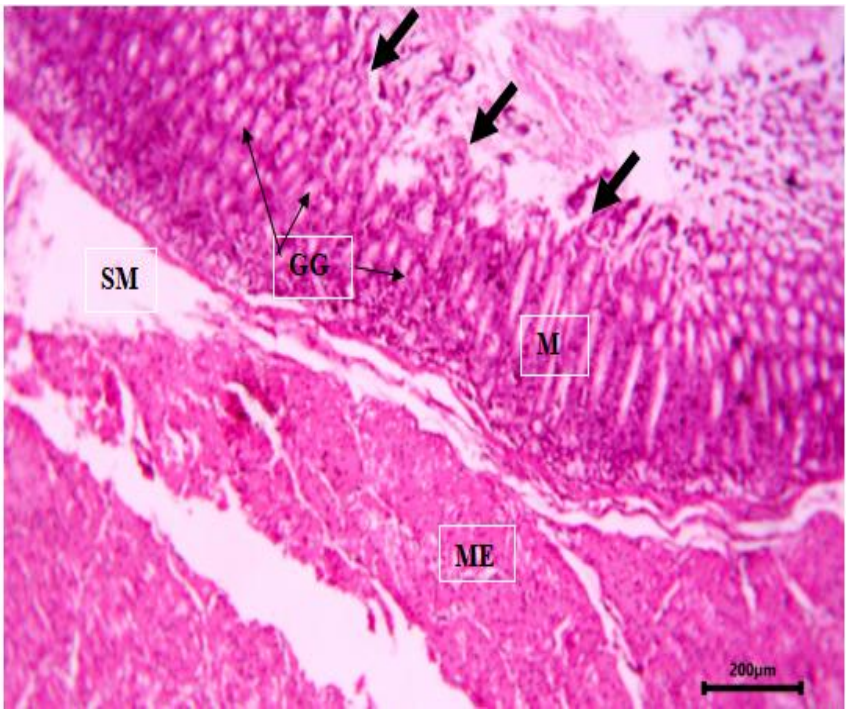

Figure 2: Photomicrograph of the indomethacin (40 mg/kg) only group H&E, x100

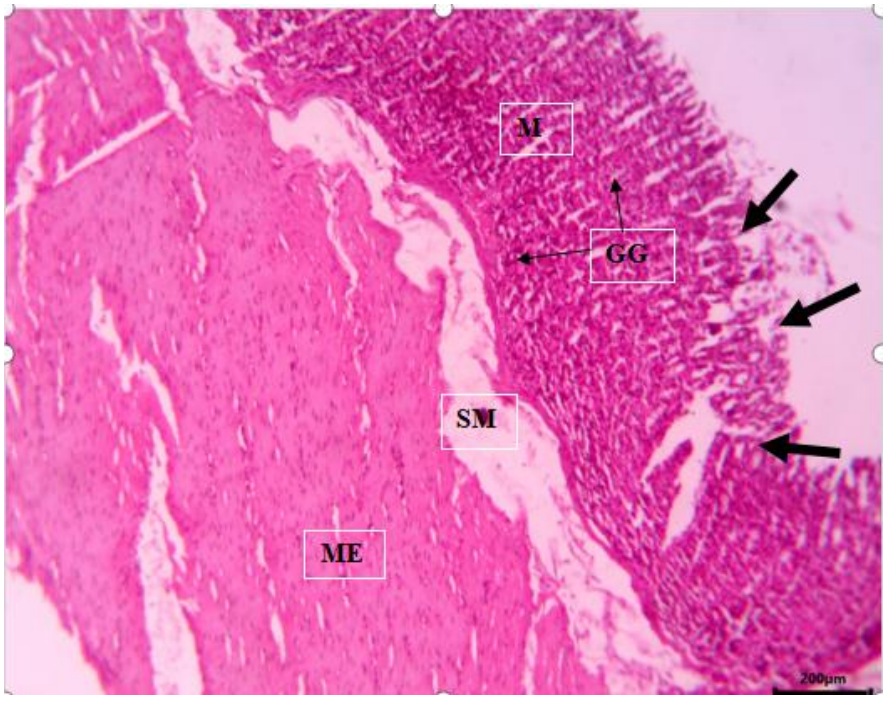

Figure 3: Photomicrograph of omeprazole (20 mg/kg) treated group H&E, x100

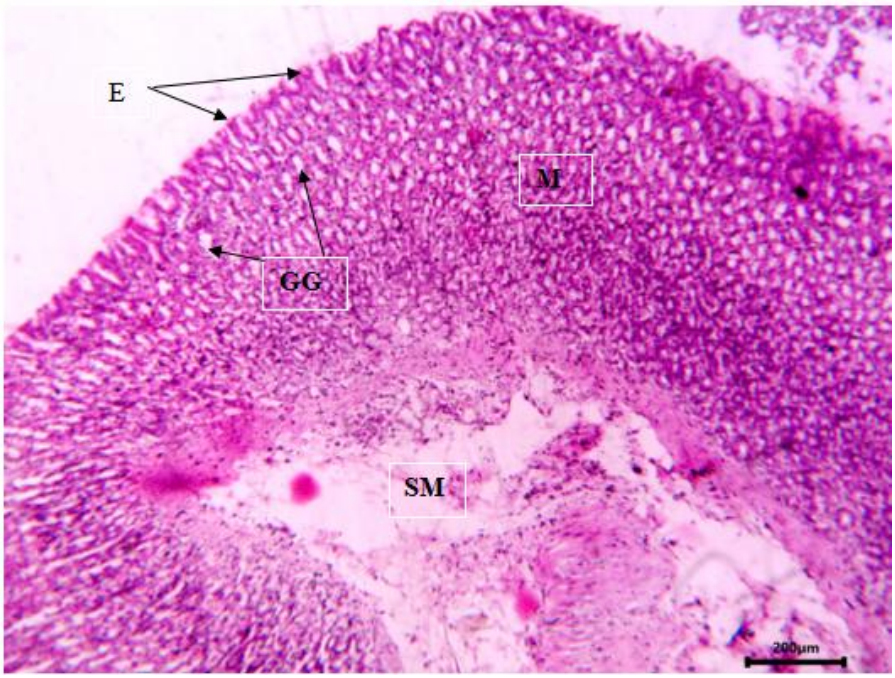

Figure 4: Photomicrograph of fraction (100 mg/kg) treated group H&E, x100

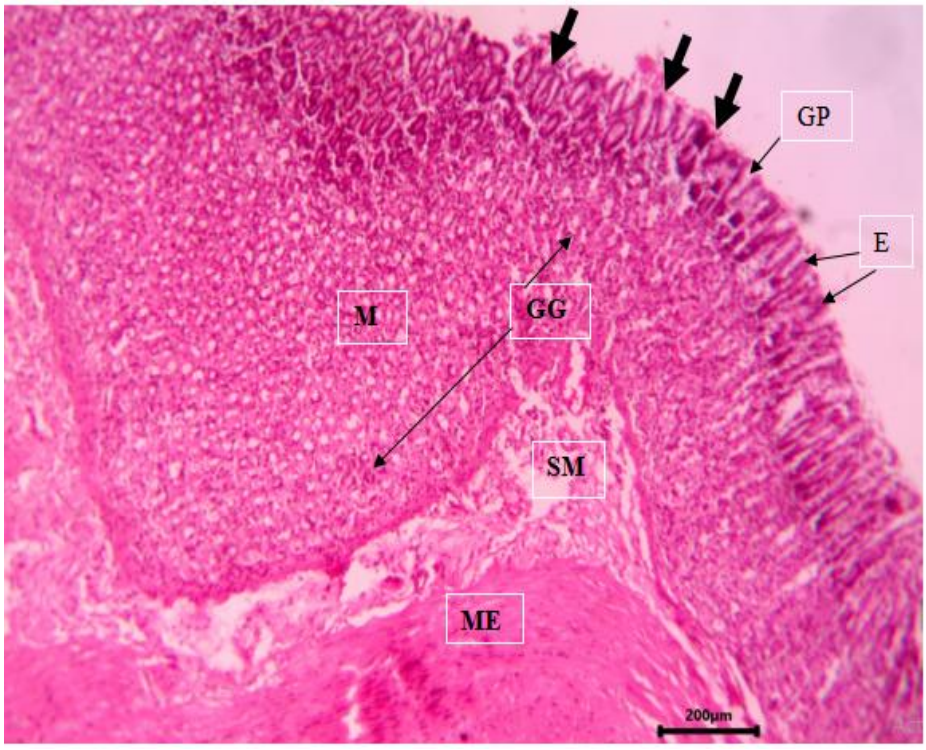

Figure 5: Photomicrograph of fraction (200 mg/kg) treated group H&E, x100

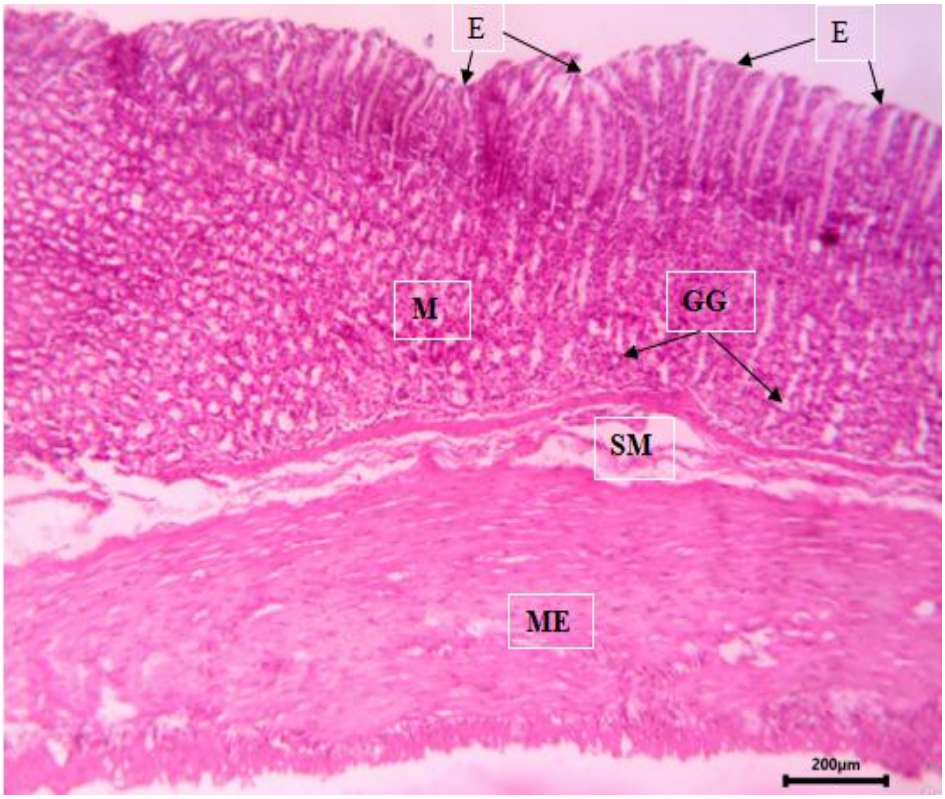

Figure 6: Photomicrograph of fraction (400 mg/kg) treated group H&E, x100

Discussion

The scourge of peptic ulcer disease is worsened in developing countries like Nigeria because of bad leadership, evidenced by so much hardship in the system with the attendant stress and poor environment, which have been observed as causative/predisposing factors in the development of PUD. Health facilities, especially in rural areas are riddled with PUD patients who lack the economic power to afford drugs for PUD. These drugs are not without their side effects and adverse reactions. This is also compounded by some of the drugs being fake or under dosed which is characteristic of corruption in developing countries. There is also the issue of access especially in the rural areas. Many resort to the use of ethno-medicine. This study, therefore, investigated the curative effects of n-hexane fraction of *D. guineense* on PUD. Chime *et al* (2025) posited that *D. guineense* could have pharmacological and other uses with LD_{50} of more than 5000mg/kg. However, N-hexane fraction of *D. guineense* was employed in this study. N-hexane is non-polar therefore accommodating lipophilic phytochemicals such as steroids and terpenoids as was observed in the phytochemical analysis. The *in vitro* antioxidant results showed significant reduction in MDA values p and a significant increase in SOD values for the treatment group, however there was no significant changes in the values of GSH and CAT for the treatment group which is at variance with other works (Agu *et al* 2023). These observations are likely as a result of different phenolic compounds and terpenoids present in N-hexane fraction of *D. guineense.* Different authorities have posited the ability of phenolic compounds to donate proton which leads to the scavenging of free radicals, reactive oxygen and inhibition of lipid peroxidation and its cytoprotective potential (Edeoga and Erieta, 2001). The findings in this study are in consonance with earlier reports (Fahmy *et al.,* 2015; Suheryani *et al*., 2019) that reported elevated levels of MDA in the gastric tissue following administration of indomethacin. Also, the findings of this study support previous studies which found that the levels of SOD were significantly decreased in the gastric tissue of rats treated with indomethacin but significantly increased in the rats treated with their study fractions (Sabiu *et al*., 2015; Alkushi and Elsawy, 2017; Jambi and Khattab, 2019). However, there was a significant decrease in total and free acidity for at lower doses This implies that N-hexane fraction at some doses have a neutralization effect probably due to the presence of terpenoids and steroids. This study is in agreement with the results of Obidike *et al*., (2024) who reported a significant decrease in free acidity by methanol extract and ethyl acetate fraction *D. guineense.* PH showed similar trend with a significant decrease between the

induction only group and the treatment group. The results of this study are at variance with the findings of Obidike *et al*., (2024) on *D. guineense* leaf extract and fractions where there was an increase in pH values.

Conclusion

The n-hexane fraction of *Dialium guineense* exhibits significant potential as an antiulcerogenic agent and may offer an alternative therapeutic option for the treatment of peptic ulcer disease.

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