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**Esther Oluwatoyin Agbaje**
*Department of Pharmacology, College of Medicine, University of Lagos, University Rd, Lagos, P.M.B. 12003, Nigeria*

**Yewa Peace Doe**
*Department of Pharmacology, College of Medicine, University of Lagos, University Rd, Lagos, P.M.B. 12003, Nigeria*

**Correspondence:**
Department of Pharmacology, College of Medicine, University of Lagos, University Rd, Lagos, P.M.B. 12003, Nigeria

# Gastric and duodenal antiulcer effects of aqueous bark extract of *Dialium guineense* Wild. (Fabaceae) and the possible mechanisms in laboratory models

Esther Oluwatoyin Agbaje, Yewa Peace Doe

## ABSTRACT

The plant *Dialium guineense* (DAG) has been claimed by local users, to be effective in the treatment of peptic ulcers, especially, when taken as an aqueous decoction. The present study assessed the antiulcer activity of the plant, as well as explored the possible mechanisms of action of the herbal drug, aside identifying some of the various phytoconstituents, which could be responsible for its antiulcer activity. Different ulcerogens (ethanol 99.9 %, indomethacin 50 mg/kg, cysteamine 400 mg/kg, glacial acetic acid) and the pylorus ligation-induced ulcers were used to induce acute and chronic ulcers, with doses of 100, 300 and 750 mg/kg DAG and the standard drugs relative to each model, while assessing drug activity through ulcer scoring and comparing it with both the negative and positive controls. The extract, which has an $LD_{50}$ of 1584.89 mg/kg when administered intraperitoneally, recorded a significant ($p<0.05$) antiulcer effect in all the models used in the study. Similarly, in the pylorus-ligated group, DAG compared effectively with atropine (1 mg/kg) and ranitidine (100 mg/kg), the standard antagonists of the secretagogues- carbachol and histamine employed in the study. The herbal drug produced a significant reduction in gastric juice volume, as well as in the free and the total acidity. The results suggest that DAG possesses a significant antiulcer property through cytoprotective and antisecretory actions, and it could be projected that the presence of secondary metabolites such as tannins, saponins and flavonoids could be responsible for its ulcer protective and healing property. The study therefore validates the folkloric use of DAG in the treatment of peptic ulcer.

**Keywords:** Peptic ulcer, *Dialium guineense*, Mice, Rats, Extract.

## INTRODUCTION

Peptic ulcers are defects in the gastrointestinal mucosa that extend through the muscularis mucosae and they persist as a function of the acid or peptic activity in gastric juice. Peptic ulcer disease (PUD) is an important cause of morbidity, and health care costs estimate of expenditures related to work loss, hospitalization, and outpatient care (excluding medication costs) are $5.65 billion per year in the United States [1].

The epidemiology of PUD largely reflects the epidemiology of the two major aetiological factors, *Helicobacter pylori* infection and the use of nonsteroidal anti-inflammatory drugs (NSAIDs). In the developed world, *H pylori* incidence has been slowly declining over the past 50 years and NSAID use has increased. This has resulted in a decline in duodenal ulcers (almost always associated with *H. pylori* infection) [2] and an increase in gastric ulcers (the main site of ulcers caused by NSAIDs).

Peptic ulcer remains common worldwide, especially in the developing world where *H. pylori* infection is highly prevalent [3]. Incidence of peptic ulcer increases with age and gastric ulcers peak in the fifth to seventh decade, while in duodenal ulcers, it is usually 10 to 20 years earlier. Both sexes in humans are similarly affected.

Complications of PUD vary in frequency geographically; in the United States, hemorrhage (73 %) is the most common complication of PUD, followed by perforation (9 %), and obstruction (3 %) [4]. The mortality rate from complications of PUD is over 10 times that of acute appendicitis or acute cholecystitis. Perforation has the highest mortality rate, followed by obstruction and hemorrhage; by contrast, a 13-year review of all surgical procedures for peptic ulcer complications at a Nigerian hospital found that obstruction was the most common complication (56 %), followed by perforation (30 %), and hemorrhage (10 %) [5]. Some regional factors that may account for these differences include the rates of NSAID consumption, prevalence of *H. pylori* infection, and the distribution and extent of gastritis. Therapeutic management now includes the early use of high-dose intravenous proton pump inhibitors (PPIs) and treatment to eradicate *H. pylori* [6]. Three classes of drugs shown to have a direct effect on *H. pylori* are antibiotics, bismuth salts and proton pump inhibitors. Most treatment regimens combine

agents from two or even all the three drug classes, because of the difficulty in eradicating the bacterium. Consequently, patients with active peptic ulcer might require a 6-week dosing of acid suppression with histamine $H_2$-receptor antagonist, and where combination therapy is implicated, poor adherence to drugs by patients as well as adverse effects due to long –term use have both constituted a setback to effective therapy [7]. The latter has constituted one of the major challenges facing pharmaceuticals, as far back as late 20th century.

Over the past decades, researchers have aimed at identifying and validating plant-derived substances for treating various diseases and ailments [8]and interestingly, more than 25 % of modern medicines are directly or indirectly derived from plants [9].

The plant *Dialium guineense* (DAG) Wild. (Family Fabaceae, sub-family Caesalpinioideae), grows in dense Savannah forests, shadowy canyons and gallery forests. It is native to Benin Republic, Burkina Faso, Cameroon, Central African Republic, Chad, Cote d'Ivoire, Equatorial Guinea, Ghana, Guinea, Guinea-Bissau, Liberia, Mali, Niger, Nigeria, Sao Tome et Principe, Senegal, Sierra Leone, Sudan and Togo [10]. It is called "Awin" (Yoruba, Western Nigeria), 'Icheku'' (Igbo, Eastern Nigeria), Tsayirarkurm (Hausa, Northern Nigeria), Velvet tamarind or black tamarind (English) and Tamarinier noir (French) [11]. DAG is used as part of various concoctions in the treatment of diverse ailments including ulcer, stomach aches, malaria, jaundice, heamorrhoids, bronchitis and wound among the different tribes in West Africa [12] and also as chewing stick (local tooth brush) among Nigerian populace. Presence of saponins in the plant is presumed to add to the cleaning effect of teeth and at the same time prevents caries and plaques on the teeth of users [13].

A tree of 30 m high with a densely leafy crown; leaves sometimes finely hairy, with a common stalk 5-13 cm long, usually with whitish flowers and fruits, more or less circular and flattened, up to 2.5 cm in diameter; densely velvety, black with a brittle shell enclosing one seed (or exceptionally 2), embedded in a dry, brownish, sweetly acidic, edible pulp.

Apart from its medicinal values, DAG serves as good firewood and could also produce charcoal. Furthermore, the wood, which is light brown in colour, is hard, durable, and heavy and is used for making vehicles, houses and flooring [14].

Among the scientific documentations on DAG are its stem bark's significant analgesic activity [15], its astringent antifungal property [16] and its efficacy in treating nasopharyngeal infections. Also noteworthy, is the molluscidal effect of its fruit [17].

The main focus in the study was to assess the antiulcer property of the aqueous stem bark extract of DAG in acute and chronic ulcers, while comparing it with appropriate standard drugs in each of the models employed, as well as speculating possible mechanisms of its antiulcer activity.

## MATERIALS AND METHODS

### Drugs

Misoprostol (BejingZizhu Pharmaceuticals, China),Omeprazole (Parmax Pharmaceuticals, India), Atropine (Sigma Chemical, CO., USA), Ranitidine (SKG Pharmaceuticals,London), Indomethacin (GeneithPharmaceuticals,China), Cysteamine HCL (Sigma Chemical, CO., USA).

### Animals

Healthy albino mice (20 – 25 g) and rats (120 – 200 g) of both sexes used, were obtained from the Laboratory Animal Centre of the College of Medicine of the University of Lagos, Idi-Araba, Lagos, Nigeria. The animals were maintained under standard laboratory conditions and were fed the pelleted rodent diet (Ladokun Feeds PLC, Ibadan-Oyo State, Nigeria), along with clean drinking water. They were all kept in clean plastic cages in a room with controlled 12 h light and dark cycle, while they acclimatized for a period of one week and were fasted for 18-24 h prior to experimentation. All protocols of handling and managing the animals followed the USA Guidelines as approved by the United States National Institute of Health (NIH) guide for Care and Use of laboratory animals and recommendation of IASP [18].

### Plant material

The bark of the plant obtained from a forest at Unity Secondary School, Ibadan, Oyo State, was identified by Mr T.K. Odewo in the Department of Botany and Microbiology, University of Lagos, Lagos-Nigeria, as *DIALIUM GUINEENSE* WILD with voucher number-LUH 3242.

### Extraction of plant

Fresh bark was collected, sun dried and weighed consecutively for 3 days until a constant weight was obtained. A quantity of the plant material, 150 g was chopped into tiny bits, pulverised and boiled in 800 ml of distilled water on a hot plate at 70 $^0$C for 30 min. The extract, left at room temperature for 72 h while shaking intermittently to facilitate extraction was decanted, filtered through a white muslin cloth and placed in an oven at 45 $^0$C to dry. The dried extract was scraped, weighed and stored airtight in dry sample bottles and kept in the freezer until ready for use.

### Acute Toxicity Study

Toxicity of the plant was determined in albino mice, through both intraperitoneal (i.p.) and oral routes [19]. The fasted animals were randomly divided into five groups (n = 5) and administered i.p. with doses 837 - 2930 mg/kg of DAG; the last group was given 5000 mg/kg oral treatment.

All the animals were closely observed for behavioural changes during the first two hours post therapy, and toxic or lethal symptoms were recorded. Monitoring of animals for any delayed toxic manifestations continued for the next 14 days.

### Phytochemical screening

The extract suspended in distilled water at a concentration of 100 mg/ml, was screened [20] for the following active constituents:

- Tannins
- Phlobatannin
- Saponins
- Flavonoids
- Cardiac glycosides
- Steroids
- Anthraquinones
- Alkaloids

### High Performance Liquid Chromatography (HPLC) analysis of water soluble Vitamins in DAG

Preparation of reference standard solutions

*Ascorbic acid (Vitamin C)*

Source: USP Reference Standard

Ten milligram of Vitamin C standard was weighed and dissolved in 10 ml of distilled water to obtain 1000 mcg/ml concentration. From the 1mg/ml-stock solution obtained, 0.25 ml was pipetted and 4.75 ml of distilled water added to obtain a concentration of 50mcg/ml. Twenty microlitre of this solution was injected for HPLC analysis.

*Preparation of DAG sample*

A standard procedure of preparation was followed. One milligram of the fine powdered sample was weighed into a sample bottle and dissolved in 5 ml distilled water. The solution was allowed to stand for 5 min after which 5 ml distilled water was again added. The solution was then placed on an orbit shaker for 8 h in order to ensure that all analyte contained in the different matrices of the bark are dissolved. The solution was then kept in the refrigerator overnight, and thereafter, filtered using Millipore filter (0.45mm) after which 2 ml of the filtrate was dissolved in 3 ml of distilled water. 20 microlitre of the solution was also injected for HPLC analysis.

*Chromatographic conditions*:
VITAMIN C STANDARD (40mcg/ml)
Column: ZORBAX SB CB 75×4.6,5 µm
Mobile Phase: METHANOL:BUFFER (20:80%)
Temperature: AMBIENT
VWD: 265nm
Flow rate: 0.7ml/min

**Induction of Ulcers in experimental animals**

*Ethanol-induced gastric ulcer*
Animals fasted for 24 hours were randomly allotted to 5 treatment groups (n= 6) and were pre-treated orally as follows:
Group 1 - Distilled water (10 ml/kg)
Group 2 – Extract (100 mg/kg)
Group 3 – Extract (300 mg/kg)
Group 4 – Extract (750 mg/kg)
Group 5 – Misoprostol (200 ug/kg)

One hour post treatment, ulcer was induced in rats through intragastric administration of one millilitre of 99.9% absolute ethanol. One hour after ulcer induction, animals were humanely sacrificed and the stomach excised and cut along the greater curvature [21]. The specimen was rinsed in normal saline and macroscopic examination of ulcer lesion was done through a hand lens. The lesions were counted and scored using standard methods [22], and stomach samples were afterwards fixed in 10% formalin for histopathology.

*Indomethacin-induced ulcer*
Animals were randomly grouped as before and pre-treated orally as follows[23]:
Group 1 – Distilled water (10 ml/kg)
Group 2 – Extract (100 mg/kg)
Group 3 – Extract (300 mg/kg)
Group 4 – Extract (750 mg/kg)
Group 5 – Omeprazole (20 mg/kg)
One hour post treatment, ulcer was induced via administration of indomethacin (50 mg/kg) orally to all the animals. The animals were sacrificed 6 hours after induction and ulcer scored as before.

*Cysteamine-induced ulcers*
Duodenal ulcers were induced by administering cysteamine-HCl (400 mg/kg) orally. Animals were pre-treated as outlined above, with 20 mg/kg omeprazole as the positive standard.
All the animals were sacrificed and the duodena excised and cut along the antimesentric side [24]. The duodenal ulcer score and ulcer index were determined.

*Pylorus ligation-induced ulcers*
Animals were randomly separated into 8 groups (n=6).
Group 1 – Distilled water (10 ml/kg)
Group 2 – Histamine (20 mg/kg)
Group 3 – Ranitidine (50 mg/kg) plus histamine
Group 4 – Extract (300 mg/kg) plus histamine
Group 5 – Carbachol (4 µg/kg)
Group 6 – Atropine (1 mg/kg) plus carbachol
Group 7 – Extract plus carbachol
Group 8 – Extract only

A pylorus ligature was carefully performed under light ether anesthesia in fasted male rats [25]. Animals received distilled water, extract, ranitidine orally, 30 min prior to ligation and atropine subcutaneously at the moment of ligature proceeding. One hour after pylorus ligation, animals separately received histamine subcutaneously and carbachol intraperitoneally. Five hours after pylorus ligation animals were euthanized by deep anesthesia; the stomach was opened and gastric secretion collected. The volume of gastric juice supernatant was measured and the acidity determined by an automated pH meter (PHM 85, Radiometer, Copenhagen, Denmark). Ulcer lesions were counted and scored as before.

**Determination of free acidity and total acidity**
One millilitre of gastric juice was pipetted out in a 100 ml conical flask, 2-3 drops of phenolphthalein indicator was then added and titrated with 0.25 N Sodium hydroxide until all traces of pink colour disappeared and the colour of the solution turned to yellowish orange. The volume of alkali added was noted. This volume corresponds to free acidity. Titration was continued until pink colour of solution reappeared. Again the total volume of alkali added was noted, this volume corresponds to total acidity.

**Chronic gastric ulcer**

*Acetic acid-induced chronic gastric ulcer*
The animals were fasted for 24 h prior to the experiment; under light ether anaesthesia, ulcers were induced by applying glacial acetic acid (0.05 ml) over the anterior serosal surface of the stomach of the rats [26]. The incisions were then sutured and animals were allowed to recover. The animals were treated as follows.
Group 1 – Distilled water (10ml/kg)
Group 2 – Extract (100mg/kg)
Group 3 – Extract (300mg/kg)
Group 4 – Extract (750mg/kg)
Group 5 - Ranitidine (50 mg/kg)

All the drugs were administered orally once daily, for 10 days after the induction of ulcer. The rats were sacrificed on the 10$^{th}$ day, the stomachs removed and cut open along the greater curvature and were examined for lesions which were scored accordingly.

**Method of Ulcer Rating** [22]
0 = no lesion
0.5 = Haemorrhage
1 = 1-3 small lesions
2 = 1-3 large lesions
3 = 1-3 thickened lesions
4 = more than 3 small lesions
5 = more than 3 large lesions
6 = more than 3 thickened lesions

**Calculation of ulcer index**
The extent of ulceration in the excised stomach and duodena was compared to the controls in each model. The extent of ulceration was expressed as an ulcer index obtained by the average of the scores in each treatment group.

**Preparation of histology slides**
The stomach and duodenum samples fixed in formalin were prepared and stained with haematoxylin and eosin stains using standard procedures.

**Statistical analysis**

The results are expressed as mean ± SEM. Statistical difference between means were determined by one-way ANOVA followed by t-test. Significance of difference was accepted as $P < 0.05$, 0.01 and 0.001.

## RESULTS

Dry DAG gave a coarse dark brown powder, with aromatic odour and sour taste. pH was 6.7

**Acute toxicity test**
Oral route did not produce any toxic symptoms or mortality up to the dose level of 5000 mg/kg body weight in the animals, however,

mortality of 0-100 % were recorded with i.p. doses ranging from 837-2930 mg/kg DAG (Fig 1).

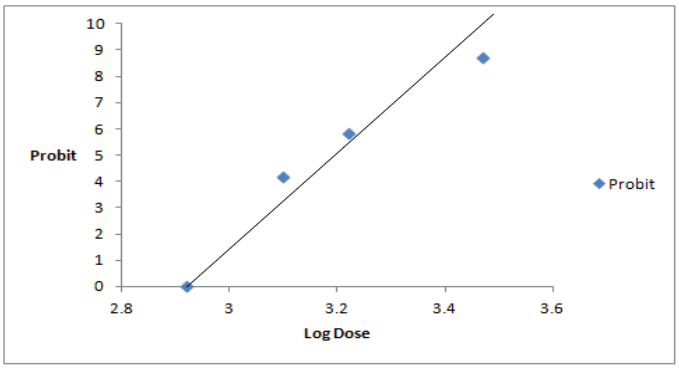


**Figure 1:** Acute Toxicity of DAG. (i.p route); $LD_{50}$ = 1584.89 mg/kg.

**Phytochemical screening**

Tannins, phlobatannins, saponins, cardiac glycosides and flavonoids were identified from DAG at 100 mg/ml concentration.

**High Performance Liquid Chromatography**

The results obtained from the HPLC analysis of the crude bark extract revealed the presence of the antioxidant Vitamin C. The concentration of Vitamin C obtained was 327.8 mg/ml.

**Ethanol induced Gastric and Duodenal Ulcers**

Pre-treatment of rats with the extract resulted in a significant ($p<0.05$; $p<0.01$; $p<0.001$) decrease in the ulcer index in a dose-independent manner. The highest percentage inhibition was exhibited by the extract at a dose of 300 mg/kg the effect which was superior to misoprostol (Tables 1). On the other hand, histology reported a better protection of the duodenum than the stomach at 300 mg/kg dose level, however, misoprostol was inferior to 300 mg/kg DGN (Tables 2 and 3; Figures 2 a, b, c and 3 a, b, c).

**Table 1:** Effect of test agents on Ethanol-induced gastric ulcer in mice

| Treatment | Mean number of ulcers | Ulcer Index | % Inhibition |
|---|---|---|---|
| DW 10 ml/kg | 4.83±0.31 | 25.92±2.04 | 0 |
| DGN 100 mg/kg | 2.67±0.95 | 5.33±2.43** | 79.42 |
| DGN 300 mg/kg | 0.83±0.54** | 0.92±0.52*** | 96.46 |
| DGN 750 mg/kg | 2.33±0.84 | 7.58±4.80** | 76.74 |
| MSP 200µg/kg | 1.17±0.62* | 5.58±4.99** | 78.46 |

The results are expressed as mean ± SEM (n=6) * P < 0.05 **P<0.01 ***P<0.001 when compared to the control.

**Table 2:** Histology of stomach in Ethanol model

| | DGN 300 mg/kg | MSP 200µg/ml | DW 10 ml/kg |
|---|---|---|---|
| Architecture | Preserved | Preserved | Preserved |
| Mucosal erosion/oedema | Healthy mucosal area but with mild oedema | Severe mucosal oedema but no erosion | Oedema present in focal areas with haemorrhage, superficial erosion |
| Chronic Inflammation (lymphocytes and plasma cells infiltrate) | Absent | Absent | Absent |
| Acute Inflammation (neutrophilic infiltrate) | Mild | Moderate | Severe |
| Submucosal oedema | Mild | Mild | Severe |
| Conclusion/Diagnosis | Mild acute gastritis | Mild acute gastritis | Severe acute erosive hemorrhagic gastritis |

**Table 3:** Histology of duodenum in Ethanol model

| | DGN 300 mg/kg | MSP 200µg/kg | DW 10 ml/kg |
|---|---|---|---|
| **Architecture** | Preserved | Preserved | Distorted mucosa |
| **Mucosal Erosion** | Not seen | Superficial mucosal erosion | Complete down to basement membrane erosion |
| **Mucosal Villus Necrosis** | Few villi are sloughed off | Few villi are necrotic | Necrotic mucosa |
| **Inflammatory neutrophils** | Few | Moderate | Severe |
| **Oedema** | Not seen | Present | Present |
| **Conclusion/Diagnosis** | Mild acute duodenitis | Moderate acute duodenitis | Acute duodenal ulceration |

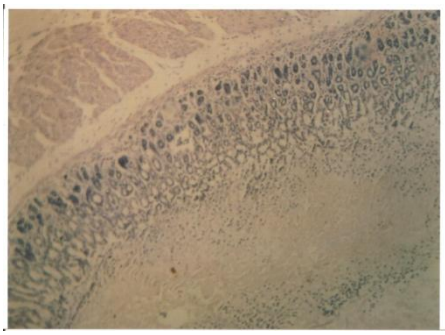

**FIGURE 2a:** Control stomach in Ethanol model (H&E × 400).
The architecture is preserved but erosion is observed in focal areas with haemorrhage resulting in severe acute erosive haemorrhagic gastritis.

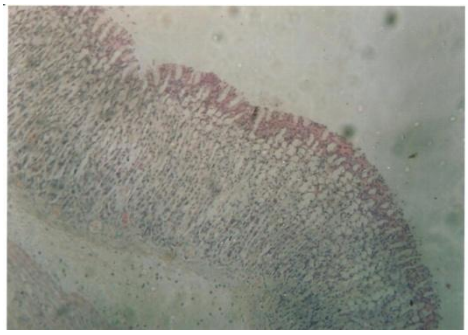

**FIGURE 2b:** *Dialium guineense* 300 mg/kgtreated stomach in Ethanol model.
Mild acute edema occurred but healthy mucosal areas (H & E × 400).

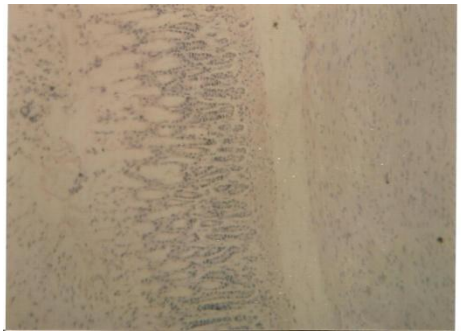

**FIGURE 2c:** *Dialium guineense* 300 mg/kgtreated stomach in Ethanol model.
Mild acute edema occurred but healthy mucosal areas (H & E × 400).

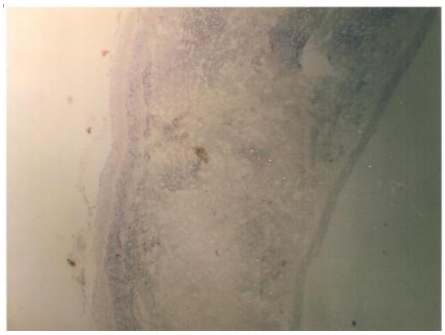

**FIGURE 3a:** Duodenum of Control (Distilled water) in Ethanol model (H & E × 400).
Complete down to basement erosion resulting in distortion of the mucosa and causing acute duodenal ulceration.

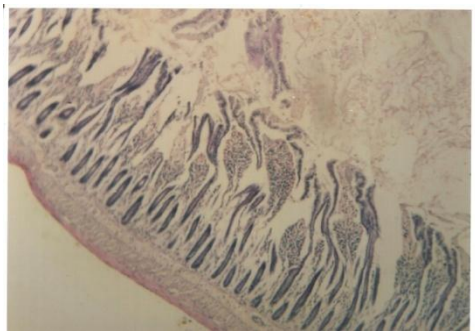

**FIGURE 3b:** *Dialium guineense*-treated duodenum in Ethanol model (H & E × 400).
The mucosa is preserved and only few villi are sloughed off resulting in mild acute duodenitis

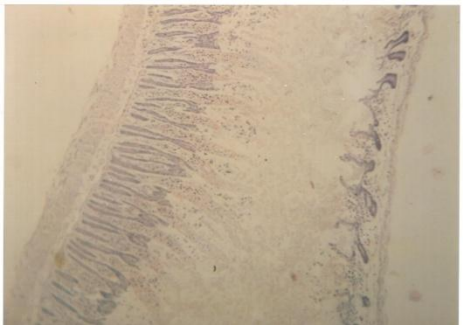

**FIGURE 3c:** Misoprostol (standard drug)-treated duodenum in Ethanol model (H & E × 400)
The mucosa is preserved but superficial mucosa erosion is observed resulting in moderate acute duodenitis.

**Indomethacin-induced ulcer**

Pre-treatment of rats with the extract (100, 300 and 750 mg/kg) produced a dose dependent protection from the indomethacin induced ulceration, as compared to control animals. However, omeprazole (20 mg/kg) produced a better inhibition than the highest dose of extract (Table 4).

**Table 4:** Effect of *Dialium guineense* on Indomethacin-induced ulcers

| Treatment | Mean number of ulcers | Ulcer Index | % Protection | Weight of mucus (g) |
|---|---|---|---|---|
| DW 10 ml/kg | 5.83±0.31 | 22.50±1.03 | 0 | 0.20±0.01 |
| DGN 100 mg/kg | 2.50±1.15 | 11.67±5.52 | 48.15 | 0.25±0.00 |
| DGN 300 mg/kg | 2.33±1.23 | 9.17±5.50 | 59.24 | 0.29±0.01 |
| DGN 750 mg/kg | 1.83±0.83* | 6.00±3.23* | 73.33 | 0.34±0.02 |
| OMP 20 mg/kg | 0.50±0.34** | 0.50±0.02** | 97.78 | 0.25±0.01 |

The results are expressed as mean ± SEM (n=6) *P<0.05 **P<0.01 when compared to the control.

**Cysteamine-induced duodenal ulcer**

Oral administration of the extract at doses of 100, 300 and 750mg/kg showed a significant (p<0.01; p<0.001) reduction in number of ulcers in a dose dependent manner compared to the control, (Table 5). However the reduction of ulcer index by the standard drug, omeprazole, was higher than that of the extract.

**Table 5:** Effect of *Dialium guineense* on Cysteamine-induced ulcer

| Treatment | Mean number of ulcers | Ulcer Index | % Inhibition |
|---|---|---|---|
| DW 10 ml/kg | 5.67±1.20 | 23.58±5.07 | 0 |
| DGN 100 mg/kg | 3.17±0.40 | 10.83±2.88* | 54.2 |
| DGN 300 mg/kg | 1.67±0.56** | 3.50±2.54*** | 84.81 |
| DGN 750 mg/kg | 1.33±0.67** | 2.17±0.49*** | 90.8 |
| OMP 20 mg/kg | 0.17±0.00*** | 0.83±0.01*** | 97.46 |

The results are expressed as mean ± SEM (n=6) *P<0.05 **P<0.01 ***P<0.001when compared to the control.

**Pylorus Ligated Model**

Effects of standard drugs histamine, carbachol, ranitidine, atropine, and DAG on gastric volume, free acid, total acid, pH and ulcer index in pylorus ligated rats were studied.

The herbal drug when used alone, recorded a significantly reduced ulcer index, compared with control and the secretagogues, histamine and carbachol treated groups. Efficacy of DAG was much reflected when given together with the secretory agents as it reduced the ulcer index for each of the agents in a more superior trend than the standard antagonists (Table 6). Reduction in gastric juice volume, free and total acidity were also observed in the extract treated group.

**Table 6:** Effect of *Dialium guineense* on Pylorus-ligated ulcer

| Treatment | Mean number of ulcers | Ulcer Index | % Inhibition | Average volume of Gastric juice (ml) | Average pH | Free Acidity (mEq/l) | Total Acidity (mEq/l) |
|---|---|---|---|---|---|---|---|
| DW 10 ml/kg | 2.17±0.18 | 3.33±0.17 | 0 | 3.00±0.20 | 2.18±0.49 | 23.14±1.23 | 54.36±1.07 |
| DAG 300 mg/kg | 0.50±0.09 | 0.67±0.12* | 79.88 | 0.63±0.07 | 2.83±0.15 | 14.24±0.36** | 43.53±0.19 |
| HST 20 mg/kg | 4.83±0.31 | 5.17±0.56 | 0 | 4.00±0.30** | 0.13±0.00 | 36.05±0.15* | 65.80±0.11* |
| RAN + HST | 1.50±0.26 | 1.75±0.19 | 47.45 | 2.17±0.09*** | 1.58±0.34 | 15.07±0.13** | 30.20±0.20 |
| DAG + HST | 0.83±0.01 | 1.42±0.03 | 57.36 | 1.57±0.02*** | 1.08±0.06 | 13.18±0.07** | 25.66±0.37 |
| CCH 4µg/kg | 3.67±1.04 | 3.80±0.65 | 0 | 2.60±0.11** | 0.98±0.09 | 30.10±0.42* | 52.44±0.25* |
| ATR + CCH | 1.33±0.18 | 1.28±0.07 | 60.06 | 1.78±0.09*** | 1.28±0.36 | 16.21±0.17** | 33.27±0.16 |
| DAG + CCH | 1.17±0.03 | 1.02±0.05 | 62.46 | 1.08±0.04*** | 1.35±0.31 | 15.30±0.10** | 32.38±0.16 |

The results are expressed as mean ± SEM (n=6)
*P<0.05 ** P<0.01 *** P<0.001 when compared to the control.
RAN= Ranitidine, HST= Histamine, CCH= Carbachol, ATR = Atropine, DAG = *Dialium guineense*, DW = Distilled water

**Acetic acid-induced chronic ulcer**

The extract at different doses produced significant ($p<0.01$; $p<0.001$) decrease in ulcer index compared to the control. The highest dose of the extract (750 mg/kg) showed the least ulcer index relative to the standard in this model (Table 7); the antiulcer action of the extract in this model is therefore dose dependent.

**Table 7:** Effect of *Dialium guineense* on Acetic acid model

| Treatment | Mean number of ulcers | Ulcer index | % Inhibition |
|---|---|---|---|
| DW 10 ml/kg | 4.17±0.65 | 5.17±1.42 | 0 |
| DAG 100 mg/kg | 0.67±0.11*** | 1.58±0.30** | 69.44 |
| DAG 300 mg/kg | 0.50±0.03*** | 0.42±0.02*** | 91.88 |
| DAG 750 mg/kg | 0.17±0.00*** | 0.25±0.01*** | 95.16 |
| RAN 50 mg/kg | 0.67±0.05*** | 0.83±0.11*** | 83.95 |

The results are expressed as mean ± SEM (n=6)
*P<0.05 ** P<0.01 *** P<0.001 when compared to the control.

## DISCUSSION

Peptic ulcer refers to the group of ulcerative disorders of the gastro intestinal tract involving principally, the most proximal portion of the stomach and the duodenum, which is commonly due to the effect of acid and pepsin [27].Stomach lesions are located preferentially along the small curvature in the transition zone between the body and the antrum, whereas in the duodenum those lesions are located in the bulb [28].

The present study investigated the effect of DAG on gastric and duodenal ulcers. The plant extract was effective in healing both acute and chronic peptic ulcers, and its efficacy was greater than the standard drugs misoprostol and ranitidine, while it compared well with omeprazole.

Ethanol-induced and indomethacin-induced gastric ulcers were employed to study the cytoprotective effect of DAG. Ethanol induces gastric lesion, due to its corrosive effect on the gastric mucosa, which it rapidly penetrates, causing cell and plasma membrane damage, leading to increased membrane permeability to sodium and water. It also produces massive intracellular accumulation of calcium, which represents a major step in the pathogenesis of gastric mucosal injury. This leads to cell death and exfoliation in the surface epithelium. Efficacy of antiulcer activity of the plant as recorded suggests its cytoprotective potential, which was superior to misoprostol, the standard drugs used (Tables 2-3; Figures 2 and 3).

Indomethacin produces erosions and ulcers in the stomach due to the inhibition of prostaglandin synthesis [29]. The gastric cytoprotective agents are effective in preventing ulcers induced by indomethacin. Similar to the effect recorded in ethanol induced gastric ulcers, DAG was effective in reducing ulcer index and increasing the mucus content. However, unlike in the ethanol model, where 300 mg/kg produced the best effect, a dose-dependent activity was recorded in the indomethacin model, with the greatest effect recorded by 750 mg/kg, and which compared effectively with 20 mg/kg omeprazole. The finding further projects the extract's antiulcer effect could be consequent to its mucosal strengthening or mucous secretion property or by increasing luminal prostaglandin levels [30].

Cysteamine-induced duodenal ulcer in rat resembles the condition in humans, both pathophysiologically and histologically. Cysteamine hydrochloride inhibits the alkaline mucus secretion from the Brunner's glands in the proximal duodenum and stimulates the rate of gastric acid secretion. Gastric emptying is also delayed and serum gastrin concentration is increased [31]. The extract produced a significant and dose dependent anti-ulcer effect with a dose of 750mg/kg of the

extract exhibiting the highest percentage inhibition, which also compared well with Omeprazole.

Pylorus ligation induced ulcer is one of the most widely used methods for studying the effects of drugs on gastric secretion. Agents that decrease gastric acid secretion and/or increase mucus secretion are effective in preventing the ulcers induced by this method, since when levels of acid overwhelm mucosal defence mechanisms; the consequence is ulcer formation [32]. The ligation of pyloric end of the stomach causes accumulation of gastric acid in the stomach, leading to the development of ulcers at the site. Ranitidine, atropine and DAG significantly decreased the secretion of gastric aggressive factors, free acidity, and total acidity, as well as the volume of acid secreted. The extract's antisecretory effect is elucidated to be via histaminergic and cholinergic mechanisms; however, its inhibitory activity is greater on carbachol.

Application of glacial acetic acid (0.05ml) on the serosal surface of the stomach produced deep penetrating gastric ulcer that resembles human peptic ulcer disease, especially in the healing process; this model therefore, is quite useful for studying the effect of drugs on the healing of peptic ulcers [33]. Extract of DAG was effective in facilitating gastric ulcer healing in this model, the effect which was better than ranitidine.

Phytochemical analysis of the bark of DAG revealed the presence of flavonoids, saponins and tannins. Flavonoids possess antiulcer and gastroprotective activity [34], also, saponinsand tannins have been reported to exhibit potent protective effects on ethanol and indomethacin-induced gastric mucosal lesions in rats [35-37]. Since flavonoids saponins and tannins have been shown to be present in the extract, it is possible that these constituents may be implicated in the antiulcer activity of DAG. It is noteworthy to report the antiinflammatory and wound healing properties of DAG (unpublished work), which could have also attributed to its profound antiulcer activity reported in this study.

High performance liquid chromatography (HPLC) analysis of the crude extract revealed the presence of antioxidant Vitamin C, which could also account for its antiulcer activity by mopping up oxidants that constituted the cause of ulcer.

Finally, this study has reported the efficacy of DAG in duodenal and gastric ulcers as well as in both the acute and chronic types also. Possible mechanisms of action have been speculated as involving cytoprotective, antisecretory and antioxidant pathways. Furthermore, the decoction is a relatively safe drug as reflected in the acute toxicity study. Further studies are required to validate the mechanisms of action and identify the active constituents in DAG.

## CONCLUSION

The investigations carried out showed both the relative safety and efficacy of aqueous bark extract of *Dialium guineense*for both prevention and therapy of peptic ulcers. It could however be inferred that *Dialium guineense* possesses cytoprotective, antisecretory and antioxidant properties. Further studies need to be conducted to identify the active component of DAG and elucidate the exact mechanisms of action.

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