Innovations

Does Methanol Extract of Dialium guineense Protect against Gastro-enteric Ulcer in Albino Wistar Rats?

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Abstract: *The shortcomings of synthetic drugs are probably going to be more obvious in near future. The study investigated the anti-ulcerogenic properties of D. guineense with the view to having a good and desirable agent in tackling gastro-enteritis. Twenty four rats were randomly distributed into six groups as follows. Group 1: Normal control, Group 2 Induction only, Group 3 Treatment with 20mg/kg bw omeprazole + induction, Groups 4-6: Treatment (100, 200 and 400 mg/kg bw respectively of crude methanol extract of Dialium guineense) + Induction. The study lasted for 15 days. Induction was done after two weeks of administration using indomethacin 40mg/kg bw. Eight hours after the induction the rats were sacrificed and the stomach and duodenum were harvested. The phytochemical result showed the presence of reducing sugar, glycosides, alkaloids, flavonoids, tannins, phenolics, among others in various quantities. The in vitro antioxidant activity suggested that the crude methanol extract had inhibitory activity against DPPH·, NO· and TBARS. The results of the phase's one and two median lethal dose showed neither deaths nor signs of acute toxicity. The results of the mean ulcer indices and percentage ulceration demonstrated that treatment groups were significantly ($P<0.05$) lower compared to induction only. The result of percentage inhibition showed that 200gmg/kgbw had the highest ulcer inhibitory activity with 91.74%. There was significant increase in antioxidant activity of treated groups compared to induction. The mechanism of action showed reduction in acidity by the sample. Histology result revealed that the sample has anti-ulcerogenic activity by cytoprotective potency.*

Key words: *Ulcer, antioxidant, histology, rats Dialium guineense*

1.0. Introduction

The integrity of the gastro-enteric portion of the gastro-intestinal tracts cannot be over-emphasized. Gastritis, obviously leads to the inflammation of the epithelial cells of the stomach. Helicobacter pylori, hyperacidity, foods low in fibre, pro-oxidant foods and nutrients, among other factors, have been implicated in heightening gastritis. Proton pump inhibitors antacid, antimicrobial agents (against H. pylori) are the key concerns of therapeutic agents with respect to peptic ulcer. The shortcomings of synthetic drugs are probably going to be more obvious in recent years Gnanaraja et al., (2014). The xenobiotic nature of these principles abound. The genetic proximities of biomolecules to that of humans cannot be over-emphasized. This has necessitated a continued search for natural compounds that are relatively more compatible with the human genome and with lesser side effects.

The choice of solvent is crucial for successful phytochemical extraction. N-hexane is best suited for non-polar compounds. For polar compounds, other solvents like methanol or ethanol are generally preferred. Good extraction efficiency was also found with methanol as extraction solvent ($p < 0.05$) for both solid-liquid (7.9%) and ultrasound (5.7%) methods. Methanol was considered the best solvent to extract phenols using the ultrasound method. Methanol is frequently used for phytochemical extraction because it's a strong, polar solvent that can efficiently dissolve a wide range of phytochemicals, including alkaloids, flavonoids, and glycosides. Its relatively low boiling point also makes it easier to remove from the extract during evaporation, which is a crucial step in processing Ragunathan et al., (2019)

The use of methanol for making plant extracts in the field of drug designing is based on its ability to efficiently extract a wide range of phytochemical compounds from plant material. Methanol is a commonly used solvent in laboratory settings due to its ability to solubilize both polar and non-polar compounds, making it suitable for extracting a diverse array of plant constituents. It is important to note that the use of methanol for extraction is not intended for direct consumption or application to humans. During the extraction process, the methanol is typically evaporated or removed from the extract to obtain the concentrated phytochemical compounds. The resulting extract can then be further processed, purified, and evaluated for its potential medicinal properties in drug discovery and development.

Although the toxicity of methanol at high doses is well established, less is known about potential adverse effects from lower levels of exposure over a long period of time, which often is the case with methanol-containing herbal extracts (International Programme on Chemical Safety 2022).

Phytochemicals are naturally occurring bioactive compounds found in plants, often giving them their color, aroma, and flavor. They are not considered essential nutrients but are believed to contribute to human health by offering protection against various diseases. Phytochemicals are isolated from plants. They are useful

and effective in this modern era that mutations of microorganisms are rampart in addition to different refractory diseases Siddiqui and Moid (2022). Phytochemicals act as antioxidants, protect against cellular damage, and may reduce the risk of chronic diseases like cancer and heart disease. Some phytochemicals have anti-ulcer potencies (Ramasubramania raja and Babu 2011). In essence, phytochemicals are a diverse group of plant-based compounds that can contribute to a healthier diet and potentially reduce the risk of various diseases (Mukheet 2019).Siddiqui and Moid (2022) stated that a chunk of the world's population make use of natural medicine in primary health care. Phenolics are said to be the most various and chemically diverse phytochemicals. They are antioxidants Saxena et al., (2013). Tannins have been reported to possess anti-inflammatory, antibacterial, free radical scavenging and haemostatic potencies Siddiqui and Moid (2022). Flavonoids, saponins, gums and mucilages and tannins were some phytochemicals that have been reported to have anti-ulcer potency Ramasubramania raja and Babu (2011).

Peptic ulcer is reported to be the most common disorder of gastro-intestinal tract resulting mainly as a result of an imbalance between the gastric defensive and aggressive factors. It is said to be more prominent in industrialized, civilized and developed countries. Use of non-steroidal anti-inflammatory drugs has been implicated in its etiology alongside smoking, alcohol consumption and stress Mukheet et al., (2019). Other precipitating factors are consumption of hot and spicy food, intake of hard fibrous diet, rotten food and excessive gastric secretions Mukheet et al., (2019).

Some factors act in different ways to prevent, heal or mitigate peptic ulcer. They range from adequate blood flow to the epithelial cells to the secretion of prostaglandins which is one of the products of the pathway that non-steroidal anti-inflammatory drugs try to inhibit. Other factors are 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). 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).

Dialium 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. They are a source of iron, magnesium and copper. Dialium guineense has been reported to have efficacy against peptic ulcer when taken as aqueous decoction Agbaje and Doe (2015). Peptic ulcers are defects in the gastrointestinal mucosa that extend through the muscularis mucosae and they persist as a result of the acid or peptic activity in

gastric juice Agbaje and Doe (2015). The report by Obidike et al., (2024) suggested that D. guineense stem bark showed substantial effect in decreasing ulcer-precipitated inflammation. Their investigations demonstrated that the plant possessed antioxidant potency in ulcer-induced albino rats. The leaves of D. guineense have been reported to possess anti-ulcerogenic potential (Ezeja et al., 2011). It belongs to the family of Fabaceae and it is also known as black velvet or velvet tamarind (Besong et al., 2016). It flowers and the fruit is red, flattened and circular (Assiki et al., 2022).

2.0 Materials and Methods

The plant leaves were collected from a natural habitat in Ibagwa Aka in Igbo-Eze South Local Government Area of Enugu State Nigeria. The sample was shade dried and pulverized.

2.1.1 Instruments/Equipment and Reagents

Different instruments were used ranging from refrigerator to magnetic stirrer, centrifuge, spectrophotometers, PH meter, test tubes syringes, vannula among others. DPPH, $FeCl_3$, $K_3[Fe(CN)_6]$, egg yolk, vitamin C, Folin Ciocalteau' Reagent among others were the reagents used. They were of analytical grade and products of Qualikem, Sigma Aldrich, among others.

2.1.2 Extraction of Crude Extract of Dialium guineense

The pulverized sample was marcerated with 80 % methanol for 72 hours. Muslin cloth was used to remove the chaff while filtration was done using Whatmann No 1 filter paper. Magntic stirrer was used to concentrate the sample to have the crude extract.

2.2 Phytochemical Analysis

The qualitative and quantitative analyses were done according to the methods of Harbone, 1973 and Trease and Evans 1989.

2.3 In vitro Antioxidant Determination

The in vitro antioxidants assayed were 1,1-diphenyl 2-picrylhydrazyl radical (DPPH.), Ferric reducing/antioxidant power (FRAP), Nitric oxide radical scavenging assay (NO.) and Thiobarbituric acid reactive species (TBARS) according to these methods of Benzie and Strain, 1996., Banerjee et al., 1996., Sreejayam and Rao 1997 and Gyamfi, et al., 1999,

2.4 The Determination of Median Lethal Dose (LD_{50}) of Dialium guineense

Mice were used for the median lethal dose determination. The median lethal dose was done according to Lorke's method (1983). Two phases were involved namely phase 1 and phase 2.

The phase one

Groups	Dosage	Observation for Possible death(s)
1	10mg/kg bw	
2	100mg/kg bw	
3	1000 mg/kg bw	

Observations were made for any sign of toxicity. After twenty-four hours observations were made for any possible death(s). Then the phase two followed

Phase Two

Groups	Dosage	Observation for Possible death(s)
1	1600 mg/kg bw	
2	2900 mg/kg bw	
3	5000 mg/kg bw	

Observations were made for any sign of toxicity. After twenty-four hours observations were made for any possible death(s).

2.5 Experimental Design

Albino Wistar rats were used for the main experiment. Twenty four rats were randomnly distributed into six groups as follows

Group 1 Normal Control
Group 2 Induction only (40mg/kg bw indomethacin)
Group 3 20 mg/kg bw omeprazole standard drug + Induction
Group 4 100 mg/kg bw MEDG + Induction
Group 5 200 mg/kg bw MEDG + Induction
Group 6 400 mg/kg bw MEDG + Induction

The study lasted for 15 days. Induction was done after two weeks of administration. Eight hours after the induction the rats were sacrificed and the stomach and duodenum were harvested. The stomach was ligated using a thread at the pylorus. The duodenum was taken for histological examination while the stomach was cut at

the greater curvature, rinsed through using clean water and carefully pinned for gross morphological examinations and scorings for possible ulceration or not.

2.6 In Vivo Antioxidants Assay

Catalase activity was assayed using the method of Aebi (1983). The product of lipid peroxidation (MDA) was estimated using the method of Wallin et al., (1993). The superoxide dismutase activities were determined using the method of Xin et al., (1991).

2.7 Some Stomach Contents Determinations

The stomach contents were checked for the negative logarithm of hydrogen ion concentration (PH), free acidity, total acidity, nitric oxide radical concentration and total carbohydrates level as suggested by

2.8 Histopathological Examination

The histological examinations of the stomachs and duodenum of albino wistar rats were done in accordance with the method of Drury et al., (1967).

2.9 Statistical Analysis

The statistical analysis was done using SPSS version 23.0. Data wer expressed as mean±SD. Analysis of variance (ANOVA) was determined using one way ANOVA. The respective mean values were separated using Duncan zceee multiple test. Test for significance was set at $P<0.05$.

3.0 Results

3.1 The Result of the Qualitative and Quantitative Phytochemical Compositions of Crude Methanol Extract of Dialium guineense

The qualitative phytochemical result showed the presence of reducing sugar, glycosides, alkaloids, flavonoids, tannins, phenolics, among others in various degrees. The qualitative phytochemical result indicated that total phenolic compounds were significantly ($P<0.05$) higher than other phytochemicals. Flavonoids and alkaloids were significantly ($P<0.05$) higher than other phytochemicals as shown in Table 1.

Table 1: Showing the Qualitative and Quantitative Phytochemicals of Crude Methanol Extract of Dialium guineense

Phytochemicals	Qualitative	Quantitative (mg/100g)
Glycosides	**+**	**38.80±0.40^{a}**
Reducing sugar	**+++**	**1827.54±208.67^{b}**
Saponin	**ND**	**ND**
Alkaloids	**++**	**135.00±13.50^{a}**
Flavonoids	**+++**	**1708.44±123611^{b}**
Tannins	**+**	**47.20±0.88^{a}**
Total phenolics	**+++**	**8612.90±58.34^{c}**
Steroids	**+**	**3.07±0.13^{a}**
Terpenoids	**++**	**41.43±1.96^{a}**

Superscripts that are the same were considered non-significant (P>0.05) down the column while those that were different were considered significant (P<0.05).

3.2 The Result of In vitro Antioxidants Activities of Crude Methanol Extract of Dalium guineense

The in vitro antioxidant activity suggested that the crude methanol extract had significant (P<0.05) inhibitory activity against 1,1-diphenyl 2- picrylhydrazyl radical (DPPH·) concentrations 15.63- 500 µg/ml scavenged DPPH radical effectively. For ferric reducing /antioxidant power (FRAP), there was significant (P<0.05) ability of the crude methanol extract of Dialuim guineense to reduce iron (111) to iron (11) as evidenced by the non-concentration –dependent power shown in Table 2.

Nitric oxide radical (NO.) demonstrated that the cruder methanol extact of Dialuim guineense scavenged NO. low activities. 31.25µg/ml showed significant (P<0.05) increase in inhibition of NO. compared to 15.63 µg/ml. 62.50 and 250 µg/ml indicated significant ((P<0.05) decrease in inhibitory activity compared to 31.25 µg/ml. With respect to thiobarbituric acid reactive species (TBARS) the crude methanol extract of Dialium guineense showed various concentration-dependent inhibition of lipid peroxidation. 500 µg/ml demonstrated the highest activity with 35.42 % compared to other concentrations.

Table 2: Showing the In vitro Antioxidant Models of Crude Methanol Extract of Dialium guineense

Conc µg/ml	DPPH. (% Inhib)	%FRAP (AAE)	NO. (% Inhib)	TBARS (% Inhib)
15.63	98.53±2.52^{a}	107.25±5.47^{a}	13.57±6.89^{a}	16.79±4.47^{a,b}
31.25	100.00±0.00^{a}	100.00±9.48^{a}	26.85±2.97^{b}	22.32±2.88^{b}
62.5	100.00±0.00^{a}	92.03±6.91^{a}	13.81±3.05^{a}	17.46±7.70^{a}
125	100.00±0.00^{a}	90.10±15.27^{a}	18.59±2.36^{a,b}	13.47±0.00^{a}
250	100.00±0.00^{a}	91.31±11.93^{a}	12.33±0.12^{a}	19.43±5.86^{a,b}
500	100.00±0.00^{a}	99.52±7.75^{a}	21.35±8.41^{a,b}	35.42±0.00^{c}

Superscripts that are the same were considered non-significant (P>0.05) down the column while those that were different were considered significant (P<0.05).

3.3 The Results of Median Lethal Dose (LD_{50})

The results of the phase's one and two showed neither deaths nor signs of acute toxicity.

3.4 The Results of the Mean Ulcer Indices, Percentage Ulceration and Percentage inhibition of Ulcer of Albino Wistar Rats Treated with Crude Methanol Extract of Dialium guineense

The results of the mean ulcer indices and percentage ulceration demonstrated that treatment groups were significantly (P<0.05) lower compared to induction only. The result of percentage inhibition showed that 200gmglkgbw had the highest ulcer inhibitory activity with 91.74% followed by 100mglkgbw (79.89) and 400mglkgbw that had 63.36% as shown in Table 3.

Table 3: Showing the Ulcer Parameters of Albino Wistar Rats Treated with Crude Methanol Extract of Dialium guineense

Groups	Mean Ulcer	% Inhibition of ulcer	% Ulceration
1	3.70±1.8^{b}	-	100.00±1.95^{b}
2	1.45±0.82^{a}	60.06	39.94±0.82^{a}
3	0.73±0.36^{a}	79.89	20.11±0.4^{a}
4	0.30±0.08^{a}	91.74	8.26±0.08^{a}
5	1.28±0.9^{a}	63.36	36.64±0.97^{a}

Superscripts that are the same were considered non-significant (P>0.05) down the column while those that were different were considered significant (P<0.05).

1 Induction only (40mg/kg bw indomethacin)
2 Standard (20mg/kg bw omeprazole)+ Induction
3 Treatment (100mg/kg bw n-hexane fraction of D. guineense) + Induction
4 Treatment (200mg/kg bw n-hexane fraction of D. guineense) + Induction
5 Treatment (400mg/kg bw n-hexane fraction of D. guineense) + Induction

3.5The Result of the In Vivo Antioxidants Activities of Albino Wistar Rats Treated with Crude Methanol Extract of Dialium guineense

The result of the malondialdehyde indicated that there was significant (P<0.05) increase in MDA concentration of group 2 (induction only) compared to the normal control. On the other hand, there was non-significant (P<0.05) differences of MDA levels of treatment compared to induction only group.

The result of reduced glutathione (GSH) showed that there was non-significant (P>0.05) increase in GSH of group 2 compared to group 1. However 200mg/kg bw of treatment showed significant (P<0.05) increase in GSH compared to the normal group and a non-significant (P>0.05) increase in concentration compared to group 2. Superoxide dismutase activities of 200 and 400 mg/kg bw of treatment were significantly (P<0.05) higher compared to induction only group. Catalase activities of treatment were significantly (P<0.05) higher compared to the normal control.

Table 4: Showing the Serum Antioxidant Parameters of Albino Wistar Rats Treated with Crude Methanol Extract of Dialium guineense

Groups	MDA (mg/dl)	GSH (mg/dl)	SOD IU/L	CAT (IU/L)
1	$0.74\pm0.16^{a,b}$	0.88 ± 0.42^{a}	11.43 ± 0.08^{d}	1.05 ± 0.34^{a}
2	$2.035\pm0.98^{c,d,e,f}$	$1.47\pm0.30^{a,b}$	10.71 ± 0.44^{a}	$2.69\pm0.45^{b,c,d}$
3	$2.39\pm0.91^{e,f}$	$1.54\pm0.55^{a,b}$	$11.08\pm0.04^{a,b,c}$	$1.97\pm0.73^{a,b,c,d}$
4	2.49 ± 0.63^{f}	$1.60\pm0.59^{a,b}$	$10.99\pm0.09^{a,b,c}$	3.36 ± 1.54^{d}
5	$1.54\pm0.44^{a,b,c,d,e,f}$	$2.07\pm0.90^{b,c}$	$11.11\pm0.10^{b,c,d}$	$1.67\pm0.41^{a,b,c}$
6	$1.5\pm0.34a^{a,b,c,d,e,f}$	$1.52\pm0.58^{a,b}$	$11.12\pm0.47^{b,c,d}$	$2.90\pm0.79^{c,d}$

Superscripts that are the same were considered non-significant (P>0.05) down the column while those that were different were considered significant (P<0.05).

1 Normal control
2 Induction only (40mg/kg bw indomethacin)
3 Standard (20mg/kg bw omeprazole)+ Induction
4 Treatment (100mg/kg bw n-hexane fraction of D. guineense) + Induction
5 Treatment (200mg/kg bw n-hexane fraction of D. guineense) + Induction
6 Treatment (400mg/kg bw n-hexane fraction of D. guineense) + Induction

3.6 The Result of some Stomach Parameters Determined to Ascertain the Possible Mechanisms of Action of Anti-ulcerogenic Potential Crude Methanol Extract of Dialium guineense Using Albino Wistar Rats

The result of the concentrations of the stomach total carbohydrates of treatment groups were significantly (P<0.05) lower compared to the induction only group and normal control. On the other hand nitric oxide radical concentrations of the treatment groups were non-significantly (P>0.05) different compared to normal control and induction only group.
The results of free acidity and total acidity demonstrated that there was a signiifcant (P<0.05) reduction in free acidity and total acidity of treatment groups compared to induction only group. With respect to negative logarithm (PH) of the treatment roup, there was a non-sigficant (P>0.05) increase compared to induction only. However, there was a significant (P<0.05) decrease in PH level of the crude extract and omeprazole compared to induction only as shown in Tables 5 and 6.

Table 5: Showing the Gastro-Parameters of Albino Wistar Rats Treated with Crude Methanol Extract of Dialium guineense

Groups No.	(µM/µl) Free acidity	(µM/µl) Total acidity	(µM/µl) Total carbohydrates	(mg/100g)
1	0.552±0.46^{a}	0.040±0.02^{d}	0.056±0.02^{b}	92.06±8.97^{b}
2	0.690±0.41^{a}	0.160±0.02^{e}	0.241±0.08^{c}	157.17±39.12^{c}
3	0.422±0.23^{a}	0.018±0.00^{a,b,c}	0.040±0.02^{a,b}	5.98±0.55^{a}
4	0.682±0.50^{a}	0.013±0.00^{a,b,c}	0.030±0.01^{a,b}	17.45±5.27^{a}
5	1.041±0.66^{a}	0.016±0.01^{a,b,c}	0.036±0.02^{a,b}	19.62±1.52^{a}
6	0.171±0.09^{a}	0.010±0.00$^{a,b.c}$	0.017±0.01^{a,b}	5.22±2.31^{a}

Superscripts that are the same were considered non-significant (P>0.05) down the column while those that were different were considered significant (P<0.05).

1 Normal control
2 Induction only (40mg/kg bw indomethacin)
3 Standard (20mg/kg bw omeprazole)+ Induction
4 Treatment (100mg/kg bw n-hexane fraction of D. guineense) + Induction
5 Treatment (200mg/kg bw n-hexane fraction of D. guineense) + Induction
6 Treatment (400mg/kg bw n-hexane fraction of D. guineense) + Induction

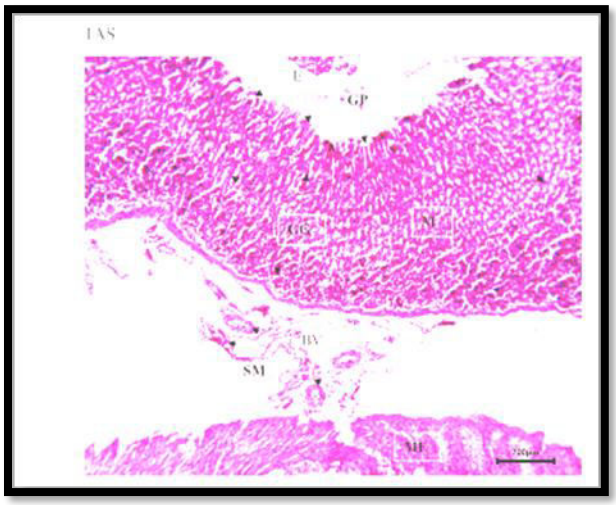
Table 6: Showing the Gastro-Parameter (PH) of Albino Wistar Rats Treated Crude Methanol Extract of Dialium guineense

Groups	PH
1 Normal control	3.83±0.88[a]
2 Induction only (40mg/kg bw indomethacin)	4.15±1.10[a]
3 Standard (20mg/kg bw omeprazole)+ Induction	4.28±0.33[a]
4 Treatment (100mg/kg bw n-hexane fraction of D. guineense) + Induction	4.60±0.93[a,b]
5 Treatment (200mg/kg bw n-hexane fraction of D. guineense) + Induction	5.10±0.65[a,b]
6 Treatment (400mg/kg bw n-hexane fraction of D. guineense) + Induction	4.18±0.05[a]
n-Hexane fraction only	7.20±0.00[c]
Omeprazole only	6.20±0.00[b,c]

Superscripts that are the same were considered non-significant (P>0.05) down the column while those that were different were considered significant (P<0.05).

3.7 The Result of the Histopathological Photomicrograph of the Stomach of Albino Rats Induced with Peptic Ulcer but Treated with Crude Methanol Extract of Dialium guineense Leaves

The result of the histopathology demonstrated that the photomicrograph of a section of albino rat as seen in group one showed normal stomach architecture. There were intact gastric mucosa, sub-mucosal and muscularis externa layer. Also, there was intact mucosal columnar epithelium. The gastric pits and glands were well defined. There were no pathological changes seen. On the contrary the stomach architecture of induction only was significantly distorted. There was shedding and ulceration in gastric epithelium and gastric pit. Group three demonstrated mild shedding and ulcer in gastric epithelium. Treatment with various concentrations of the sample restored the stomach architecture to normal as seen in Plates 1-6.



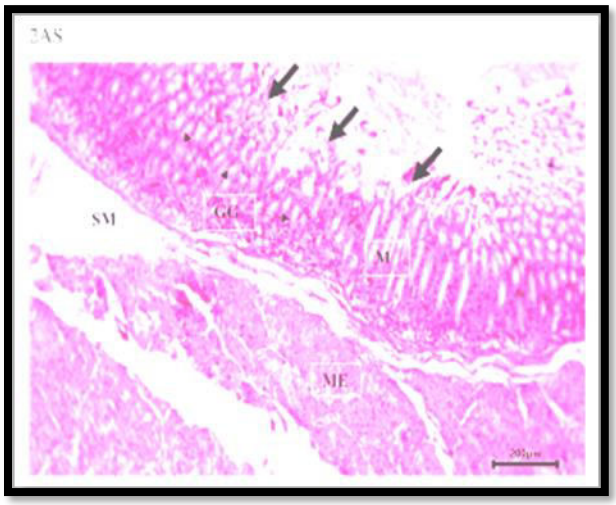
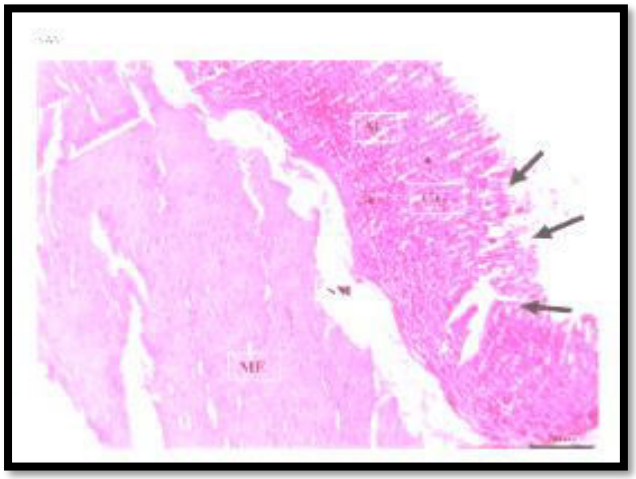


Plate 1 Group 1 Stomach photomicrograph, Plate 2Group 2 Stomach photomicrograph



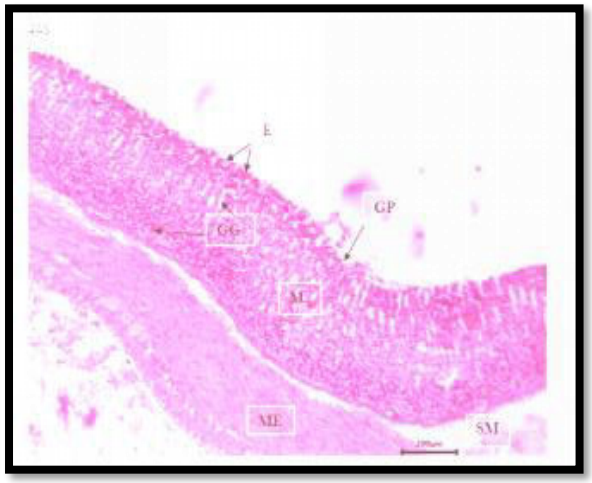
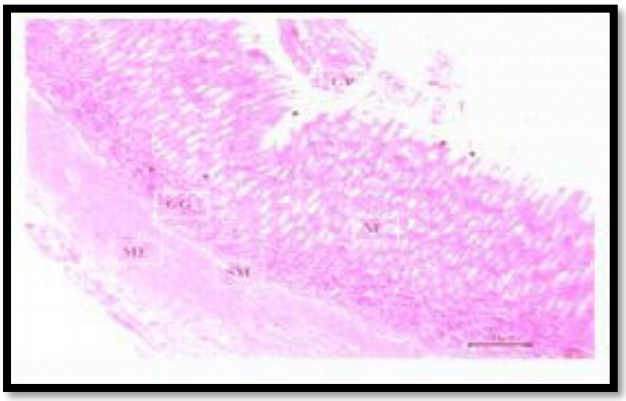


Plate 3 Group 3 Stomach photomicrograph, Plate 4 Group 4 Stomach photomicrograph



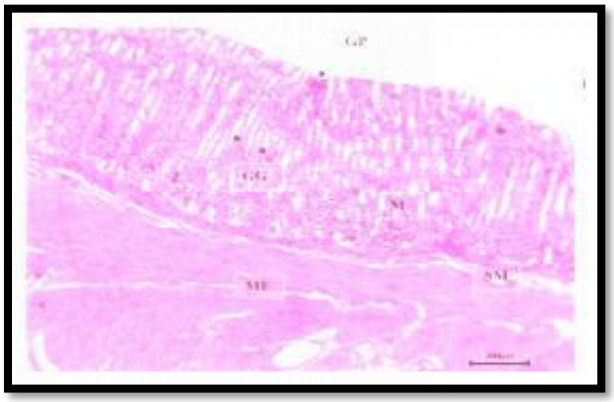


Plate 5 Group 5 Stomach photomicrograph, Plate 6 Group 6 Stomach photomicrograph

4.0 Discussion

The integrity of the gastro-enteric portion of the gastro-intestinal tracts cannot be over-emphasized. Gastritis, obviously leads to the inflammation of the epithelial cells of the stomach. Helicobacter pylori, hyperacidity, foods low in fibre, pro-

oxidant foods and nutrients, among other factors, have been implicated in heightening gastritis. Proton pump inhibitors antacid, antimicrobial agents (against H. pylori) are the key concerns of therapeutic agents with respect to peptic ulcer. The shortcomings of synthetic drugs are probably going to be more obvious in recent years Gnanaraja et al., (2014), hence the continued search for natural remedies for peptic ulcer. The study investigated the anti-ulcerogenic properties of D. guineense with the view to having a good and desirable agent in tackling gastro-enteritis.

The extraction employed eighty-percent methanol. Methanol has both polar and non-polar constituents. Its polarity index is 5.1 relative to pentane that has polarity index of 0.0. This solvent system ensured that non-polar and polar phyto-constituents of the plant could be extracted Ragunathan et al., (2019). It could enable the extract to act reasonably better in both the extracellular spaces, intracellular mileu and at the cell membranes.

The phytochemicals extracted reflected the benefits of employing a solvent system that would extract both polar and non-polar constituents. Reducing sugars and glycosides were extracted. Terpenoids, however was moderately present. Total phenolics, including flavonoids and tannins were also extracted. Phytochemicals have various pharmacological activities that have been attributed to them Siddiqui and Moid (2022). Flavonoids have cyto-protective effect, antioxidant, anti-cancer effects, among other biological activities. All flavonoids have a common biosynthetic origin and possess phenylchromane skeleton (Laszlo, 2017). Resonance in the aromatic A ring is common in all categories of flavonoids. Stability of the ring, absorption of energy and resistance of easy oxidation by this structural system might lend credence to the antioxidant potencies of flavonoids. Besides a good number of flavonoids have hydroxyl group. The ability of these compounds to donate proton (since most flavonoids are water soluble) contribute to free radical scavenging abilities of flavonoids. In addition, some flavonoids exhibit isoprenylation. This process introduces double bonds in the isoprene unit added. The double bonds act as antioxidant. They help in minimizing oxidative abstraction of proton and in absorbing different quanta of energy which could easily activate some biological molecules thereby making them more prone to be easily oxidized by pro-oxidants.

Total phenolics can delay the rate of oxidation, scavenge free radicals especially effect of electron leakage from electron transport chain. The presence of these phytochemicals in D. guineense seemed to suggest that the extract had pharmacological potency. The median lethal dose showed that the sample is relatively safe up to 5000 mg/kg bw.

The in vitro antioxidant results indicated that crude methanol extract of D. guineense scavenged DPPH radical. DPPH mimics amino acids in that it is a nitrogen-based radical. Ability of any principle to scavenge DPPH radical could be extrapolated to mean that the principle can also prevent the oxidation of amino group in proteins

thereby preventing denaturation of the protein Saxena et al., (2013). If the protein were to be a trans membrane protein, maintaining its integrity is tantamount to aiding in the maintenance of membrane integrity and by extension cell functions. Ferric reducing/antioxidant power (FRAP) result suggested that the crude extract of D. guineense was able to reduce iron (III) to its reduced form, iron (II). Haemoglobin has iron in +2 state. Different authors have opined that D. guineense modulates the haemoglobin concentrations of albino wistar rats. This seemed to lend credence to the plants ability to reduce the rate at which free iron is oxidized from +2 state to +3 state. Children especially cherish eating the leaves of D. guineense. This practice is highly encouraged so that the ingestion will contribute in haematopoietic processes and reduction of anaemia, especially among children. Ladies that are menstruating can also make a tincture of tea with the pulverized dry leaves to ameliorate blood loss during monthly periods. Nitric oxide radical and thiobarbituric acid reactive species were moderate in activity. The moderate nature is required so as to have the benefits of moderate nitric oxide. Moderate nitric oxide inconveniences the proliferation of microbes. It also plays a good role in the protection of the epithelial cells. However, in high concentrations nitric oxide can have negative effect, hence the need to have it in moderate amount. Thiobarbituric acid reactive species captures the ability of a given extract to inhibit lipid peroxidation. There is heightened increase in frying food items currently in the modern societies. Consequently, there seemed to be a remarkable increase in gastritis as the integrity of the parietal cells of the stomach wall is highly distorted. D. guineense does not increase lipid peroxidation as indicated by the result of TBARS in this study.

The result of the antiulcer investigation indicated that the sample significantly prevented ulceration. The mean ulcer indices of treatment were much reduced compared to the induction only group. Ulcerations were more pronounced in induction only group compared to the treatment groups. The presence of substantial values of flavonoids and total phenolics may account for these observations. These phytochemicals have been reported to have antiulcer potency (Ramasubramania raja and Babu 2011). The report by Obidike et al., (2024) suggested that D. guineense stem bark showed substantial effect in decreasing ulcer-precipitated inflammation. The leaves of D. guineense have been reported to possess anti-ulcerogenic potential (Ezeja et al., 2011).

The in vivo antioxidant results suggested that the crude methanol extract of D. guineense possessed in vivo antioxidant potency. The sample did not increase oxidation relative to the normal rats. There seemed to be a corroboration of the in vitro antioxidant result with that of the in vivo antioxidant results. The phenolic compounds in the extract could account for these observations.

In furtherance of the mechanisms of action, the PH, free acidity, total acidity, nitric oxide and total carbohydrates of the stomach contentsof the albino wistar rats treated with crude methanol extract of D. guineense were investigated. The PH of

the crude extract was significantly more alkaline than the induction only group of albino rats. Thus PH of the crude caused a reduction in acidity of the stomach contents of albino rats treated with the crude methanol extract of D. guineense. The free and total acidity of the stomach contents indicated that the administration of the alkaline extract led to the neutralization of stomach acidity of the albino rats. Increased acidity is known to to increase or heighten ulceration. Hence any drug that is alkaline will at least reduce stomach acidity in ulcer patients. It seemed to be one of the mechanisms of action of the crude methanol extract of D. guineense in ameliorating peptic ulcer. Treating albino rats with the sample did not alter the nitric oxide radical. However, it did also lead to a negative outcome. The total carbohydrate result showed that mucin levels of the rats were altered. This seemed to be as a result of the antioxidant potency of the sample Saxena et al., (2013).

The histopathological results of the stomach and duodenum of albino Wistar rats suggested that the restoration of the stomach architecture corroborates the finding that crude methanol extract of Dialium guineense demonstrated cytoprotective potency. The presence of flavonoids, tannins, terpenoids and phenolic compounds might account for this. The mild ulceration observed in the standard drug suggested that while omeprazole has antiulcer potency it may not be a viable option in preventing ulcer. Besides the drug omeprazole might not be a good antioxidant and cytoprotective agent.

4.1 Conclusion

Crude methanol extract of Dialium guineense has antiulcer activity, antioxidant potency and cytoprotective potential. Therefore it could be used in treating peptic ulcer precipitated by non-steroidal anti-inflammatory drugs. The maintenance of iron in +2 state makes the sample an agent that could be tackle anaemia, especially among children. Ladies that are menstruating can also make a tincture of tea with the pulverized dry leaves to ameliorate blood loss during monthly periods.

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