Estimation of Anti-Ulcerogenic Effects of Ethanol extract and Fractions of *Dialium guineense* stem bark in Ethanol-Induced peptic ulcer in Albino Rats.

Abstract

Background/Objectives

Peptic ulcer disease is characterized by desisting in the inner lining of the gastrointestinal (GI) tract because of gastric acid secretion or pepsin. It expands into the muscularis propria layer of the gastric of the gastric epithelium. It may include the lower esophagus, distal duodenum or jejunum. The emergence and resistance of some over the counter anti-ulcer agents and its cost in developing countries have necessitate the need to optimize antiulcer actions of stem bark of *Dialium guineense* and its efficacy. This study circles to evaluate the anti-ulcerogenic potentials of ethanol extract and fractions of *Dialium guineense* stem bark in ethanol-induced ulcer in albino rats.

Material and Methods: the effects of *Dialium guineense* stem bark in ethanol-induced ulcers were determined using normal procedures. The spss version 22 was used for one way ANOVA. Analysis ($p<0.05$)

INTRODUCTION

The stomach is the major receptacle as food travels through the gastrointestinal tract (GIT) for digestion (Chan and Lau, 2021). The stomach walls are protected from erosive effects of the acid and enzymes that participate in the digestive process by mucous linings. Upon an imbalance between the digestive apparatus (enzymes and acids) and the protective mucous linings, ulcer occurs. Another major cause of ulcers is the bacterium, *Helibacter pylori* (Cover and Blasé, 2020).

Dialium guineense, commonly called *velvet temarid* or *black velvet* belongs to the family of *fabaceae.* Its leaves, bark and seed are widely used in African herbal medicine to treat many ailments, such as bronchitis, toothache, cough, bacterial, plasmodial, diarrhaeal, stomach upsets and haemorrhoidal diseases (Abu *et al.,* 2022). Available literature indicates that its stem bark has antioxidant and anti-inflammatory activities.

Peptic ulcers (ie gastric and duodenal ulcers) diseases are breaks in the gastric and duodenal mucosa of the upper gastrointestinal tract. Ulcers ranges between 3mm and several centimeters.

Peptic ulcer disease is characterized by desisting in the inner lining of the gastrointestinal (GI) tract because of gastric acid secretion or pepsin. It expands into the muscularis propria layer of the gastric of the gastric epithelium. It may include the lower esophagus.

MATERIALS AND METHODS

Collection and identification of sample: The study took place in the laboratory unit of Michael Okpara University of Agriculture, Umudike Abia State, Nigeria between August, 2023 to Febuary, 2024. *Dialium guineense* stem bark was obtained from Mercy Girls High School, Okigwe, Imo State Nigeria

The plant was identified and authenticated by Prof. G.G.E. Osuagwu of the Department of plant Science and Biotechnology, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria. A voucher specimen was kept at the herbarium in the Department of Physiology and Pharmacology, College of Veterinary Medicine.us, distal duodenum or jejunum (Talia *et al* 2022).

Preparation of Plant Materials

Fresh stem bark of *Dialium guineense* were washed with clean water and sun- dried. The dried stem bark were pulverized into fine powder, using a grinding machine.

Extraction of Plant Materials

A known quantity (1000g) of the pulverized stem bark of *Dialium guineense* was soaked in 3 litres of ethanol for 48 hours. The mixture was filtered through Whatman No 1 filter paper and the filtrate concentrated to a solid residue using rotary evaporator.

The crude ethanol extract was stored in a refrigerator (Thermocool, Nigeria) at 10°C until the time of use.

Percentage yield of the extract was calculated using the formula (Johnlouis, O.I *et al.* 2022)

$$\text{Yield (\%)} = \frac{X}{Q} \times \frac{100}{1}$$

Where:

X = Weight of dried *Dialium guineense* bark extract after extraction (2.00 g)

Q = Weight of *Dialium guineense* bark extract powdered plant material before extraction

Study design for the biochemical estimation of *Dialium guineense bark* extract:

160 mature adult albino rats were used according to the method of Michael, *et al.*,2013. The albino rats were assigned into six group of 5 rats each and were treated as follows:

Group 1	-	Normal control
Group 2	-	Negative control
Group 3	-	Received 20mg/kg body weight of Omeprazole
Group 4	-	Received 200mg/kg body weight of *Dialium guineense*
Group 5	-	Received 400mg/kg body weight of *Dialium guineense*
Group 6	-	Received 800mg/kg body weight of *Dialium guineense*

30 minutes after treatment with the extract, all the animals were administered with 1ml of ethanol via route, except the normal control. The animals were sacrificed after 1 hour following the ethanol induction. The ulcer scores were read from harvested stomachs which were opened longitudinally along the greater curvature with scissors and spread on a dissection board using a hand lens. Blood samples were collected for biochemical haematological analysis. Fresh stomach and jejunum samples were also collected and immediately transferred into cold chain and afterwards, homogenized and centrifuged to obtain supernatants which were used for biochemical analysis. The same process was followed in both acute, sub-acute ethanol and fractions for ethanol models.

Macroscopic evaluation of the stomach (ulcer)

The stomach were excised and carefully opened along the line of greater curvature to expose the walls. The stomach contents were then washed off and viewed with aid of light microscope (x100) to determine the ulcer scores using method of **Raju *et al.*, (2009).**

The ulcerative lesions were counted and scored as follows:

Normal stomach	0
Pinhole	1.0
Spot ulceration	1.5
Haemorrhage streaks	2.0
Small erosion	2.5
Large erosion	3.0
Perforation	3.5

Mean ulcer score for each animal was used to express the ulcer index. The ulcer index (U.I) was calculated by using the formula:

$$U.I = \frac{1 \times (\text{No. of lesions of grades 1}) + 2 \times (\text{No. of lesions of grade 2}) + 3 \times (\text{No. of lesions of grades 3})}{10}$$

The percentage ulcer protection was determined using the formula described by Suzik, *et al.*, 1976.

$$\text{Protection index} = 1 - \frac{\text{ulcer index with extract}}{\text{Ulcer index with distilled water}} \times 100$$

Pro-inflammatory cytokine assay

Tumor necrosis factor-alpha (TNF-α) assay

An ELISA based method was used for the assay as described by Heydayati *et al*., 2001.

Principle: The ELISA kit uses sandwich- ELISA as the method. The micro-ELISA plate provided in the kit was pre-coated with an antibody specific RAT TNF-α. Standards or samples were added to appropriate micro-ELISA plate walls and combined with the specific antibody. Then biotinylated specific antibodies and avidin Horseradish peroxidase (HPR) conjugate was added to each microplate wall and incubated. HRP conjugate appeared blue colour. The enzyme substrate reaction was terminated by adding stop solution and the solution

turned yellow colour. The optical density (OD) was then measured using spectrophotometer at wavelength of 450nm. The optical density value is proportional to TNF- α concentration.

Test preparations

Homogenized tissues were washed with 0.01m phosphate buffer solution before homogenization. The tissue homogenate was then mixed with the same concentration of buffer in ratio of 1:9 (tissue weight: volume of buffer). Homogenate was then centrifuged for 5minutes at 500nm to get supernatant. Samples and reagent were all brought to room temperature before the analysis. Stock washed buffer solution was measured out and made up of 750ml of working buffer solution using de-ionized water.

Standard working solution: stock stand solution was first centrifuged at 10,00rpm for 1 minute. 1ml of stock + 1ml of sample diluents were measured into test tubes and was mixed by turning upside down while incubating for 10minutes. This gave a standard solution of 500mg/ml. serial dilutions were then made in the gradient as follows: 5,000, 2,500, 1,250, 625, 312.5, 156.25, 78.13 and 0mg/ml

Procedure:

1. 1.00ml of standard or sample was added to each of either standard or sample. And incubated for 90 minutes at 37^0C.
2. Liquid was removed and 100ml biotinylated detection antibody, was added and incubated for 1 hour at 37^0C
3. Liquid (solution) was aspirated and washed 3 times
4. 100ml conjugated was added and incubated for 30 minutes at 37^0C
5. Solution was aspirated and washed 5 times
6. 90ml stop solution was added and optical density read immediately using micro plate reader at 540nm.
7. A graph of OD was plot against concentration for known standard concentration and concentration for sample extrapolated from graph. Duplicate readings were averaged.

Evaluation of enzymatic antioxidants

Estimation of superoxide dismutase (SOD)

Superoxide dismutase activity was assayed by the method of Arthur and Boyne (1985) as contained in Randox kit.

Principle: The method employs xanthine and xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T) to form a red formazan dye. The superoxide dismutase activity is then measured by the degree of inhibition of this reaction. One unit of SOD causes a 50% inhibition rate reduction of (I.N.T) under the conditions of the assay.

Procedure: To 0.05ml diluted sample in attest tube was added 1.7ml of mixed substance solution and mixed xanthine oxidase (0.25ml) was added. The initial absorbance was taken after 30 seconds. The final absorbance was taken after 3 minutes and units of SOD per gram haemoglobin were extrapolated from a standard curve.

Estimation of catalase

The activity of catalase was assayed by the method of Sinha (1972).

Principe: Dichromate in acetic acid was reduced to chromic acetates when heated in the presence of hydrogen peroxide with the formation of perchromic acid as unstable intermediate. The chromic acetate formed was measured at 570nm. Catalase was allowed to split H_2O_2 for different periods of time. The reaction was stopped at different time intervals by the addition of dichromate acetic acid mixture and the remaining H_2O_2 was determined by measuring chromic acetate colorimetrically.

Procedure: To 0.9ml of distilled water and 0.1ml of plasma in a test tube was added to 2ml of H_2O_2 and 2ml phosphate buffer. The reaction was initiated by adding 2ml of dichromate acetic acid reagent to 1ml portion to this mixture. Absorbance of the reaction was taken in 30 seconds interval for 2 minutes. The activity of catalase was expressed as U/ml of plasma (U-micro-moles of H_2O_2 utilized per second.

RESULTS

TABLE 1: **Effects of fractions on ulcer scores in rats**

Treatments	UN	US	UP	UI	Percentage ulcer inhibition
Normal control	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	100.00±0.00g
Ulcer control	16.33±1.53e	30.00±2.29f	100.00±0.00f	14.63±0.33g	0.00±0.00a
Omeprazole, 20 mg/kg	10.00±1.00c	19.00±1.32c	88.33±2.89c, d	11.77±0.43c, d	19.58±2.94d,e
FI, 500 mg/kg body weight	ro14.00±1.00 d	24.50±1.32e	100.00±0.00f	13.85±0.23f, g	5.33±1.57a,b
F2, 500 mg/kg body weight	9.67±0.58c	19.33±1.67c,d	85.00±5.00c	11.40±0.71c	22.07±4.82e
F3, 500 mg/kg body weight	5.67±1.53b	7.50±2.18b	71.67±2.89b	8.48±0.55b	42.01±3.78f
F4, 500 mg/kg body weight	10.33±1.53c	21.67±2.84c,d, e	91.67±2.89d, e	12.37±0.68d, e	15.47±4.67c,d
F5, 500 mg/kg body weight	12.67±1.52d	21.83±2.93c,d, e	93.33±2.89e	12.78±0.73e	12.62±4.98c
F6, 500 mg/kg body weight	13.67±1.16d	22.83±1.04d,e	95.00±0.00e	13.15±0.22e, f	10.12±1.49b,c

From table 1 above, the ulcer score count recommended that the counts of the negative control were significantly higher ($p<0.05$) when compared to the normal control group while the count of the co-treated groups were significantly ($p<0.05$) lower when compared with that of normal control. Percentage inhibition of the ulcer group is zero, thus significantly ($p<0.05$) lower than that of the normal control which had 100% protection. However, the percentage protection of the omeprazole control group (11.17%) and those of the co-treated groups were significantly ($p<0.05$) lower than that of the control.

Table 2: The Results on change in pro-inflammatory cytokines.

Treatment groups	TNF-α (pg/ml)	IL-1β (pg/ml)	PGE2 (pg/ml)	MAPK (ng/ml)	iNOs (pg/ml)
Normal control	46.70 ± 2.49^{a}	0.50 ± 0.02^{a}	40.33 ± 2.50^{a}	1.11 ± 0.04^{a}	5.59 ± 0.26^{a}
Ulcer control	83.10 ± 2.95^{d}	1.83 ± 0.24^{d}	82.33 ± 2.41^{c}	2.22 ± 0.39^{b}	10.37 ± 0.38^{d}
Omeprazole, 20 mg/kg	66.03 ± 4.13^{c}	1.27 ± 0.12^{c}	62.90 ± 2.96^{b}	1.33 ± 0.06^{a}	8.21 ± 0.51^{c}
Extract, 200 mg/kg body weight	65.90 ± 3.57^{c}	1.13 ± 0.09^{c}	60.37 ± 7.31^{b}	1.28 ± 0.06^{a}	8.77 ± 0.15^{c}
Extract, 400 mg/kg body weight	60.37 ± 4.37^{c}	0.90 ± 0.08^{b}	60.27 ± 4.95^{b}	1.23 ± 0.03^{a}	8.25 ± 0.28^{c}
Extract, 800 mg/kg body weight	54.13 ± 1.36^{b}	0.67 ± 0.04^{a}	56.40 ± 4.52^{b}	1.23 ± 0.08^{a}	7.23 ± 0.65^{b}

From the table 2 the change in pro-inflammatory cytokines, indicated that the TNF-α, showed no significant different between fractions F1, F4, F5 and F6 and omeprazole and they are significantly lower than the ulcer group. The IL-1β, PGE2, MAPK and iNOs showed significant decrease in omeprazole group and the fractions (F1, F2, F3, F4, F5 and F6) when compared to the ulcer control.

Table 3: **Effects of fractions on Antioxidant on the ethanol extract of *Dialium guineense* stem bark.**

Treatments	GSH (mg/dl)	GPx (u/mg protein)	SOD (u/mg protein)	CAT (u/mg protein)	MDA (mmol/mg protein)
Normal control	10.67±0.93c	6.13±1.43c	26.30±1.28c	21.77±2.15c	0.37±0.02a
Ulcer control	7.97±0.44a	29.17±0.59a	20.93±1.50a	16.90±0.50a	1.80±0.21d
Omeprazole, 20 mg/kg	9.10±0.15b	31.23±1.34a,b	24.17±1.02b	18.60±1.30a,b	1.09±0.08c
FI, 500 mg/kg body weight	8.88±0.16b	31.87±1.70b	22.20±1.57a,b	19.63±1.07b,c	1.18±0.08c
F2, 500 mg/kg body weight	9.20±0.21b	33.03±0.51b	23.13±1.40a,b	18.93±0.35a,b	1.07±0.11c
F3, 500 mg/kg body weight	10.03±0.24c	33.70±1.91b	23.90±0.70b	20.53±1.91b,c	0.88±0.04b
F4, 500 mg/kg body weight	8.97±0.14b	32.23±1.56b	21.43±1.45a	19.83±0.45b,c	1.04±0.15b,c
F5, 500 mg/kg body weight	9.08±0.29b	31.23±0.76a,b	21.33±1.02a	19.60±1.21b,c	1.09±0.05c
F6, 500 mg/kg body weight	8.88±0.55b	32.17±1.40b	22.13±0.29a,b	19.20±0.56b	1.12±0.03c

In the table 3 above, the GSH result showed that there is no significant different in the normal control and fraction 3 (F3) and no significant different between omeprazole, fractions (F1, F2, F4, F5 and F6) and they are significantly higher than ulcer control. The GPx showed significant different in all the fractions which showed significant increase when compared to ulcer group. SOD showed significant decrease in omeprazole and the fractions when compared to the normal control. CAT results indicated no significant different between the fractions (F1, F2, F3, F4, F5 and F6) which are significantly higher than ulcer group. In MDA, there is no different in the omeprazole group and fractions (F1, F2, F4, F5 and F6) and they are higher than the normal control.

DISCUSSION

The TNF-α suppresses gastric microcirculation around ulcerated mucosa and thus delays its healing (Hasgul, R., S 2014). The reduction of the TNF-α and PGE2 in the fractions could be an indication of suppression of ethanol-induced toxicity. It is, therefore, reasonable to suggest that the inhibition of NF-κB is the key mechanism by which the *Dialium guineense* stem bark extract causes the suppression of ethanol-induced ulceration since the expression of several pro-inflammatory cytokines including TNF-α, PGE2 and IL-1b is mainly regulated by the transcription of NF-κB (Li, W, 2013). The NF-κB is a transcription factor that plays significant roles in toxicity, such as expression of many pro-inflammatory targets including adhesion molecules, TNF-α and chemokines such as PGE2 (Mei, X., D *et al.* 2012). Thus *Dialium guineense* stem bark extract apparently caused significant ($p<0.05$) protection against ethanol-induced ulceration. This could be achieved either directly via inhibition of NF-κB target receptors such as the pro-inflammatory tumor necrotizing factor or indirectly through mopping up pro oxidants by the antioxidant properties of *Dialium guineense* stem bark extract.

The antioxidant enzymes in all body cells consist of three major classes of antioxidant enzymes which are the catalases, (CAT), superoxide dismutases (SOD) and glutathione peroxidases (GPX), all of these, play crucial roles in maintaining homeostasis in cells and their induction reflects a specific response to pollutant oxidative stress (Birben, E *et al*., 2012).

Antioxidants are assumed to help control wound oxidative stress and thereby accelerate wound healing. Since the extract caused increased production and secretion of gastric mucus, it could therefore accelerate gastric ulcer healing (Obidike, *et al.*, 2024). That was indicative of an improved mucus secretory potential of the extract and indicative of its significant role in the ulcer healing process. Healing of mucosa epithelial cells was obviously displayed by all the tested fractions, thus showing a better ulcer healing capacity when compared with the omeprazole.

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Catalase reduces oxidative stress and lipid peroxidation either by protecting the detoxifying enzymes by increasing the efficacy of Nicotineamide Dinucleotide Phosphate (NADPH) or by helping in the elimination of compounds that produce peroxidation in the cell membranes (Abd-Elkareem, M 2022). Since H_2O_2 acts as a substrate for a specific reaction that generates highly hydroxyl radicals, it is believed that the primary role of catalase in cellular antioxidant defense mechanisms is to reduce the accumulation of H_2O_2 (Htet, A.S *et al*.,2017). The reduction in catalase activity could be a result of oxidative stress leading to the depletion of the enzyme. Thus, the increase in the enzyme activity in the co-treated groups could be credited to the high antioxidant activity of different fractions of *Dialium guineense* bark extract which decreased the ROS leading to improved antioxidant activity. Increasing the CAT activity would further diminish the ROS thereby upsetting the induction of stomach ulcers and permitting healing of the ulcers.

The role of SOD is to scavenge superoxide radicals and convert them to H_2O_2 (Hayyan, M. 2016). Thus reduced SOD activity in the untreated group (ulcer control) could be an indication of oxidative stress. This agrees with the result of Nkanu *et al*., 2018, that the reduction in serum SOD activity is thought to be a result of extreme autoxidation and progressive glycation

of enzymatic proteins. However, the increase in the enzyme activity in the co-treated groups could be credited to the high antioxidant activity of the different fractions of *Dialium guineense* bark extract which weaken the oxidative stress, thereby ameliorating the ethanol mechanism for ulcer induction.

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