

Estimation of Anti inflammatory and Anti oxidant property of Ethanolic extract of *Dialium guineense* stem bark in Ethanol-Induced peptic ulcer in Albino Rats

Abstract

The hallmark of peptic ulcer illness is desisting in the gastrointestinal (GI) tract due to either pepsin or gastric acid release. It expands into the muscularis propria **layer 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. The purpose of this study is to assess the anti-ulcerogenic properties of ethanol extract and stem bark fractions from *Dialium guineense* in albino rats that have been given an ethanol-induced ulcer. When compared to omeprazole and the fractions of the groups treated with 200 mg/kg, 400 mg/kg, and 500 mg/kg body weight of ethanol extract of *Dialium guineense* stem bark, the results of TNF- α , IL- β , and PGE2 revealed a substantial rise in the ulcer group. In GPx and CAT there were significant increase in normal control when compared to the ulcer group and different fractions, this findings showed that By enhancing the effectiveness of nicotineamide dinucleotide phosphate (NADPH) or by safeguarding the detoxifying enzymes, catalase lowers oxidative stress and lipid peroxidation. Further research should be on the identification of compounds which is responsible for the anti-inflammatory and antioxidant of *Dialium guineense* stem bark.

Keywords: *Dialium guineense*, ethanol, omeprazole, ulcer

INTRODUCTION

The stomach is the major receptacle as food travels through the gastrointestinal tract (GIT) for digestion (5). 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* (6).

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 (1). Available literature indicates that its stem bark has antioxidant and anti-inflammatory activities.

Peptic ulcers (i .e 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 February, 2024. *Dialium guineense* stem bark was obtained from Mercy Girls High School, Okigwe, Imo State Nigeria Prof. G.G.E. Osuagwu of the Department of Plant Science and Biotechnology at Michael Okpara University of Agriculture Umudike, Abia State, Nigeria, identified and verified the plant. “A voucher specimen was housed in the College of Veterinary Medicine's Department of Physiology and Pharmacology herbarium., distal duodenum or the jejunum” (19).

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.

Up to the time of usage, the crude ethanol extract was kept in a refrigerator (Thermocool, Nigeria) at 10°C.

The extract's percentage yield was computed using formula (11).

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

Q 1

Where:

X = Weight of extracted, dry *Dialium guineense* bark extract (2.00 g)

Q = Weight of powdered plant material prior to extraction of *Dialium guineense* bark extract.

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

160 mature adult albino rats were used according to the method of (2). 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” [11].

Fractionation of the extract

The semi-solid ethanol (20g) was fractionated in a glass column (150cm x 15cm) packed with 200g of slurry of silica gel (30, 60, 90, mesh). The column was eluted in succession with 500ml of chloroform, petroleum ether, (petether), ethanol and methanol to obtain their respective fractions of chloroform petether fraction (PEF), ethanol fraction (EF) and methanol fraction (MF) respectively.

Pro-inflammatory cytokine assay

Tumor necrosis factor-alpha (TNF- α) assay

An ELISA based method was used for the assay as described by (22).

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 wells and combined with the specific antibody. Then biotinylated specific antibodies and avidin Horseradish peroxidase (HRP) conjugate was added to each microplate well 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

“Prior to homogenization, homogenized tissues were rinsed with 0.01 m phosphate buffer solution. Next, the tissue homogenate was combined with the same buffer concentration in a 1:9 tissue weight to buffer volume ratio. After homogenate, supernatant was obtained by centrifuging it for five minutes at 500 nm. Before the analysis, the reagent and samples were all brought to room temperature. De-ionized water was used to measure out 750ml of working buffer solution, which was then combined with stock washed buffer solution” [11].

Standard working solution: the stock stand solution was centrifuged for one minute at 10,000 rpm. One milliliter of stock and one milliliter of sample diluents were placed into test tubes, shaken, and given ten minutes to incubate. The resulting standard solution was 500 mg/ml. Following then, several dilutions were made:

Procedure:

1. 1.00ml of standard or sample was added to each of either standard or sample. And incubated for 90 minutes at 37⁰C.
2. Liquid was removed and 100ml biotinylated detection antibody, was added and incubated for 1 hour at 37⁰C
3. Liquid (solution) was aspirated and washed 3 times
4. 100ml conjugated was added and incubated for 30 minutes at 37⁰C
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 (20) 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 (18).

Principle: 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: The Results on change in pro-inflammatory cytokines.

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

From the table 1 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-1 β , 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 2: 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 body weight	9.10±0.15b	31.23±1.34a,b	24.17±1.02b	18.60±1.30a,b	1.09±0.08c
F1, 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 2 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

“TNF- α slows down the healing process by obstructing the stomach microcirculation around ulcerated mucosa (8). The fractions' lower PGE2 and TNF- α values could point to less ethanol-induced damage.. Hence, it makes sense to propose that the primary mechanism through which the stem bark extract of *Dialium guineense* inhibits ethanol-induced ulceration is the inhibition of NF- κ B, given that the transcription of NF- κ B primarily controls the expression of multiple pro-inflammatory cytokines, such as TNF- α , PGE2, and IL-1b” (12). “A transcription factor called NF- κ B is important for toxicity because it regulates the production of several pro-inflammatory targets, such as adhesion molecules, TNF- α , and chemokines like PGE2” (14). Therefore, it appears that the stem bark extract from *Dialium guineense* significantly ($p < 0.05$) protected against ulceration caused by ethanol. This could be accomplished in two ways: directly by blocking NF- κ B target receptors such the pro-inflammatory tumor necrotizing factor, or indirectly by using *Dialium*'s antioxidant capabilities to absorb pro-oxidants.

“The three main classes of antioxidant enzymes found in all body cells are glutathione peroxidases (GPX), superoxide dismutases (SOD), and catalases (CAT). These enzymes are essential for preserving cell homeostasis, and their induction signifies a particular reaction to oxidative stress caused by pollutants” (4). “The three primary groups of antioxidant enzymes found in all body cells are glutathione peroxidases (GPX), superoxide dismutases (SOD), and catalases (CAT). These enzymes are essential for preserving cell homeostasis, and their activation indicates a sAntioxidants are thought to hasten wound healing by assisting in the regulation of oxidative stress in wounds. The extract may hasten the healing of gastric ulcers because it boosted the formation and release of gastric mucus” (16). This demonstrated the extract's enhanced capacity to secrete mucus and demonstrated its important function in the healing of ulcers. All of the examined fractions clearly showed repair of mucosa epithelial cells, indicating a higher ability to heal ulcers than omeprazole.specific reaction.

“By shielding the detoxifying enzymes and boosting the effectiveness of nicotineamide dinucleotide phosphate (NADPH), or by aiding in the removal of substances that cause peroxidation in cell membranes, catalase lowers oxidative stress and lipid peroxidation” (19). As H₂O₂ serves as a substrate for a certain process that produces highly hydroxyl radicals, the main function of catalase in cellular antioxidant defense mechanisms is thought to be lowering H₂O₂ accumulation (10). One possibility for the decrease in catalase activity is that the enzyme is being depleted due to oxidative stress. Therefore, it is possible to attribute the increased enzyme activity in the co-treated groups to the strong antioxidant activity of various bark extract fractions from *Dialium guineense*, which reduced reactive oxygen

species and enhanced antioxidant activity. Increasing CAT activity would further reduce ROS, disrupting the process that causes stomach ulcers and allowing the ulcers to heal..

Superoxide radicals are scavenged by SOD, which then transforms them into H₂O₂ (9). Therefore, decreased SOD activity in the ulcer control group, which was not given any treatment, may be a sign of oxidative stress. This is consistent with the finding of (15), which suggests that severe autoxidation and increasing glycation of enzyme proteins are the likely causes of the decrease in serum SOD activity.. However, Due to the high antioxidant activity of the various fractions of Dialium guineense bark extract, which reduce oxidative stress and so improve the ethanol mechanism for ulcer development, the co-treated groups may have shown an increase in enzyme activity.

Conclusion

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. *Dialium guineense* was found to have anti-inflammatory and anti-oxidant effect which helps in the management and ameliorating of diseases like peptic ulcer.

Ethical Approval

Animal Ethic committee approval has been collected and preserved by the author(s)

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REFERENCES

1. Abu, O.D, Onoagbe I.O and Ekugum E. (2022). Nephrotoxic Evaluation of Aqueous Stem Bark Extract of *Dialium guineense* in Normal Wistar Rats. *International Journal of Pharmaceutical and Bio medical Science*. 2(9) 10.3390.
2. Aishat Imasuen, Festus Inegbedion , Chritipher Erhabor And Michael Osuide (2013): Isolation and Characterization of Castor Oil and its Utilization Potential in the Production of Polyurethane Foam. *Walailak J. Sci & Tech*. 11 (5); 421 – 427
3. Author, J.R. and Boyne, R. (1985) Superoxide Dismutase and Glutathione Peroxidase Activities in Neutrophils from selenium Deficient and copper Deficient cattle. *Life science*, 36, 1569-1575.
4. Birben, E., U.M. Sahiner, C. Sackesen, S. Erzurum and O. Kalayci, (2012). Oxidative stress and antioxidant defense. *World Allergy Organ. J.*, 5: 9-19.
5. Chan, F.K.L., and Lau, J.Y.W. (2021). Peptic ulcer disease. In: Feldman M, Friedman , Brandt LJ, eds. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease*. 11th ed. Philadelphia, PA: Elsevie: chapter 53.
6. Cover, T.L., and Blaser, M.J. (2020). *Helicobacter pylori* and other gastric *Helicobacter* species. In: Bennett JE, Dolin R, Blaser MJ, eds. *Mandell*,
7. Hasgul, R., S. Uysal, H. Haltas, S. Akyol, Y. Yuksel, A. Gurel and F. Armutcu, (2014). Protective effects of Ankaferd blood stopper on aspirin-induced oxidative mucosal damage in a rat model of gastric injury. *Toxicol. Ind. Health*, 30: 888-895.
8. Hasgul, R., Uysal, S., Haltas, H., Akyol, S., Yuksel, Y., Gurel, A. and Armutcu, F., (2014). Protective effects of Ankaferd blood stopper on aspirin-induced oxidative mucosal damage in a rat model of gastric injury. *Toxicol. Ind. Health*, 30: 888-895.
9. Hayyan, M., Hashim, M.A. and AlNashef, I.M, (2016). Superoxide ion: Generation and chemical implications. *Chem. Rev.*, 116: 3029-3085.
10. Htet, A.S., Kjøllestad, M.K. Aung, W.P. Myint, A.N.M and Aye. W.T. (2017). Lipid profiles and determinants of total cholesterol and hypercholesterolaemia among 25-74 year- old urban and rural citizens of the Yangon Region, Myanmar: A cross-sectional study. *BMJ Open*, Vol.7. (10).11-36.
11. Johnlouis, O.I, Ifeanyi, N.C. and Oluchukwu, O.P. (2022). Consequence of *Tetrapleura tetraptera* leaves on pro-oxidants, hepatic functions and histomorphology in monosodium glutamate-intoxicated rats. *Res. J. Med. Plants*, 16: 37-48

12. Li, W., Huang, H. Niu, X. Fan, T. Mu Q. and Li, H,(2013). Protective effect of tetrahydrocoptisine against ethanol-induced gastric ulcer in mice. *Toxicol. Appl. Pharmacol.*, 272: 21-29.
13. Mahmoud Abd-Elkareem , Mahmoud Soliman , Mokhless A.M., Abd El-Rahman , Nasser S. and Abou Khalil .The protective effect of *Nigella sativa* seeds against monosodium glutamate-induced hepatic dysfunction in rats. *Toxicol. Rep.*,9:147-153.
14. Mei, X., Xu, D, Zheng, Y. and S. Xu, (2012). Novel role of Zn (II)-curcumin in enhancing cell proliferation and adjusting pro-inflammatory cytokine- mediated oxidative damage of ethanol-induced acute gastric ulcers. *Chem. Inte ract.*, 197: 31- 39.
15. Nkanu, E.E., Ujong, G., Okon, V., and Bassey, I.E, (2018). *In vivo* efficacy of *Dialium guineense* fruit pulp on hemeoxygenase-1 and angiotensin converting enzyme in experimental diabetes. *Journal of Med. Plants Res.*, 12: 483-492.
16. Obidike Ikechukwu Johnlouis, Aloh Godwin Sunday, Obike Chiemeziem Adanma and Okechukwu Jane Ngozi.(2024) Evaluation of Anti-Ulcerogenic Effects of the Crude Extract and Fractions of *Dialium guineense* stem Bark in Ethanol-Induced Peptic Ulcer in Albino Rats Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Nigeria. (1) 254.
17. Raju, D., Ilango, K., Chitra, V, and Ashish.K. (2009) Evaluation of Anti-ulcer activity of methanolic extract of Terminalia chebula fruits in experimental rats) *Journal of Pharmacological and Science & Res.* Vol.1 (3), 101-107.
18. Sinha, K.A. (1972) Colorimetric Assay of Catalase. *Analytical Biochemistry*, 47, 389- 394.
19. Talia F. Malik; Karthik Gnanapandithan; and Kevin Singh. (2022). Peptic Ulcer Disease: 613-624. Preventative and Therapeutic Potential of Flavonoids in Peptic Ulcer, *National Library of Medicine.* 390 (10094).
20. Author, J.R. and Boyne, R. (1985) Superoxide Dismutase and Glutathione Peroxidase Activities in Neutrophils from selenium Deficient and copper Deficient cattle. *Life science*, 36, 1569-1575.
- 21 Mahmoud Abd-Elkareem., Mahmoud Soliman., Mokhless A. M, Abd El-Rahman., Nasser S. and Abou Khalil. (2022). Effect of *Nigella sativa* L. Seed on the Kidney of Monosodium Glutamate Challenged Rats.
22. Heydayati, M., Dugas, M. J., Buhr, K., and Francis, K. (2003,). The relationship between intolerance of uncertainty and the interpretation of ambiguous and unambiguous information. Poster presented at the Annual Convention of the Association for Advancement of Behaviour Therapy, Boston, MA.

