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Some Biochemical And Haematological Evaluation In Ethanol Extract Of *dialiumguineense* Stem Bark In Ethanol Induced Peptic Ulcer In Albino Rats.

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ABSTRACT

This study was to evaluate some Biochemical and Haematological effect of ethanol extract of *Dialiumguineense* stem bark in ethanol induced peptic ulcer in Albino rats. The stem bark of Dialiumguineense were collected at Mercy Girls High School Okigwe. Adult albino rats weighing (200-311kg) both sexes were obtained from the animal house of the Department of Zoology, University of Nigeria, Nsukka and used for the study. The animals were housed under standard conditions 12/13h light-dark cycle starting 8:00am at temperature of 25. The result of RBC, PVC and Hb and WBC showed no significant ($P < 0.05$) different in the groups treated with 200mg/kg, 400mg/kg and 800mg/kg body weight of an extract. PLT has no significant different ($P < 0.05$) in all the groups. In the MCV result, the omeprazole group, the ulcer group and groups treated with 200mg/kg, 400mg/kg, 800mg/kg body weight of an extract indicated no significant different and they are significantly($P < 0.05$) `higher than the normal group. In liver markers, the albumin, ALP and total bilirubin showed no significant different ($P < 0.05$) in the omeprazole group, and the groups treated with 200mg/kg, 400mg/kg, 800mg/kg body weight of the extract. The total protein also showed no significant different ($P < 0.05$) in the groups treated with 200mg/kg, 400mg/kg, 800mg/kg body weight of *Dialiumguineense* stem bark. This study showed that Dialiumguineense stem bark is efficacious and effective due to its phytochemical properties that have antioxidant effects and ability to heal wound.

KEY WORDS: D*ialiumguineense,* ethanol, omeprazole, ulcer.

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). Ulcers do occur or extend to other parts of the GIT, including the duodenum (duodenal ulcers), and a combination of the duodenal and stomach ulcers collectively called peptic ulcer (Talia. *et al.,* 2022).

Symptoms of ulcers include bleeding that may manifest as blood in stool, eroded stomach wall (sometimes completely), burning or gnawing central abdominal pain, indigestion, heartburn, acid reflux and general feeling of being sick.

Orthodox anti-uler drugs include cimetidine (H2-blocker) in duodenal ulcer, benign gastric ulcer, recurrent and stomach ulceration and other conditions in which reduction of gastric acid is beneficial as in meal-related dyspeptic symptom (EMDEX., 2022). Others are Esomeprasol, Fematidine, Lansoprasol, Rabeprasol, Ranitidine and Omeprazole which is a proton-pump inhibitor. All these orthodox drugs are associated with many and varied side adverse effects, including diarhoea, dizziness, Gynaecomastia, Fltulence, anorexia, headache, rashes and acute pancreatitis among others.

Consequently, attention is being directed at drugs which do not cause the afore-mentioned and other adverse effects, hence this study on the Anti-ulcerogenic potential of ethanol extract of Dialiumguinence stem bark in Ibuprofen-ulcerated Albino Rats.

Dialiumguineense, 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.

In this study, crude ethanol extract and fractions of its stem bark will be tried for their effects in reversing or ameliorating ibuprofen induced ulcer in Albino Rats and compared with cimetidine.

MATERIALS AND METHODS

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 *Dialiumguineense*
Group 5	-	Received 400mg/kg body weight of *Dialiumguineense*
Group 6	-	Received 800mg/kg body weight of *Dialiumguineense*

Collection of Plant Materials

The stem bark of *Dialiumguineense* were collected from Mercy Girls Secondary School, Okigwe, Nigeria. They were identified in the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, Abia State and a voucher specimen kept in the Herbarium.

Extraction of Plant Materials

A known quantity (1000g) of the pulverized stem bark of *Dialiumguineense* 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.

Determination of Red Blood Cell (RBC) by haematocytometry

The method of Ochei and Kolhalkar (2008) was used

Procedure: An aliquot (0.02ml) of blood added to 3.98ml of sodium citrate and mixed well. After 5 minutes, the first few drops were discarded by holding the pipette vertical and the counting chamber was charged with the fluid. It was allowed to settle for 3 minutes. By switching to low power (10x) objective, the centre large square with 25 small squares were adjusted to light and then adjusted to high power (40x) objective. The red cell in the four corners and one central square were counted.

Determination of total White Blood cell by haemocytometry

Principle: The total leucocyte count was determined following the method described by Ochei and Kolhatkar (2008). Glacial acetic acid lyses red cells while gentian violet slightly stains the nuclei of leucocytes. The blood specimen diluted 1: 20 in WBC pipette with the diluting fluid and the cells were counted under lower power microscope by using a counting chamber. The number of cells in undiluted blood was reported as the number of white cell/mm^3 of the whole blood. 1% acetic acid solution facilitates haemolysis of RBC and gentian violet stains in the nuclei of RBC.

Packed cell volume (PCV) estimation

Principle: PCV estimation was described by Ochei and Kolhatkar (2008). Blood component which possess different densities, separate into layers in correspondence to their various densities microhaematocrit reader.

Determination of haemoglobin (Hb) concentration

This was done using cyanomethgemoglobin method described by Ochei and Kolhatkar (2008).

Principle: The haemoglobin is mixed with Drabkin's solution which contains potassium ferricyanide, potassium cyanide and potassium dihydrogen phosphate. The ferricyanide, form of methaemoglobin which is converted to cynohaemoglobin by the cyanide. The cynomethaemoglobin produces a colour which is

read colorimetrically.

Assay of Aspartate aminotransferase Activity

A Randox commercial Enzyme kit according to the method of Reitman and Franklel (1957) was used to assay for the activity of aspartate amino transferase.

Principle.

This method is based on the principle that oxaloacetate is formed from the reaction below.

α –oxoglutarate + L – aspartate →AST→ L –glutamate + oxaloacetate

Assay of AlanineaminoTransferase

The activity of alanine aminotransferase was assayed by the method of Reitman and Frankel (1957) as outlined on Randos kit.

Principal:

Alanine amino transferase assay is based on the principle that pyruvate is formed from the reaction below: a

α – oxoglutarate-L-Alanine ⟶ L-glutamate _+ pyruvate

ALT activity was by monitoring the concentration of pyruvate hydrazine formed with 2, 4-dinitrophenyl hydrazine.

Assay of Alkaline Phosphatase Activity

The activity of alkaline phosphate (ALP) was assayed by the method of Klein *et al* .(1960) as outlined in DCA kit.

Principle

Serum alkaline phosphatase hydrolyzes a colourless substraphate of phenolphthaline monophosphate giving rise to palkhosphoric acid and phenolphetale in which is alkaline pH values, turns to pink whose absorbance is estimated using spectrophotometer.

Method of obtaining total albumin

Principle:

The principle of this reaction is that serum proteins react with copper sulfate in sodium hydroxide to form a violet "biuret" complex. The intensity of the violet color is proportional to the concentration of protein.

Albumin is generally measured by a dye-binding technique that utilizes the ability of albumin to form a stable complex with bromocresol green dye. The BCG-albumin complex absorbs light at a different wavelength from the unbound dye. This method may overestimate albumin by binding to other proteins. The total globulin fraction is generally determined by subtracting the albumin from the total protein. (McPherson, R.A . 1984).

Electrophoresis is the most common means of further fractionating serum proteins. In this process, protein solutions in appropriate buffered solvents are placed on a medium such as paper or starch blocks and exposed to an electrical current. Differences in their electrical charge cause the protein components to migrate at different rates toward the anode or cathode.

STATISTICAL ANALYSIS

The data obtained from the laboratory tests were subjected to one-way analysis of variance (ANOVA). Differences between means at ($P < 0.05$) were accepted as significant. The results were expressed as mean ± standard deviation (SD). This analysis was estimated using computer software known as statistical product and service solution (SPSS).

RESULT

Effect of Haematological parameters on ethanol extract of *Dialliumguineense* stem bark

From the table 1, the result of RBC showed no significant different ($P < 0.05$) in groups treated with 20mg/kg of omeprazole, 40mg/kg, and 800mg/kg body weight of the extract and the normal control. The result of PCV and Hb showed that the groups that received 200, 400 and 800mg/kg are higher than the ulcer group but no significant different in the ulcer group and the group treated with omeprazole in Hb. The WBC showed no significant different in all the treated groups and significantly lower ($P < 0.05$) than ulcer group. The result of PLT and MCV showed no different in groups treated with omeprazole, 200mg/kg, 400mg/kg and 800mg/kg body weight of the *Dialiumguineense* extract stem bark which show significant increase when compared to the

normal control. In MCHC, groups treated with 200, 400mg/kg of the extract and omeprazole group is significantly lower when compared to the ulcer group and no significant different ($P < 0.05$) in with the normal control .

Table 1: Haematological parameters

Treatment	Normal control	Ulcer control	Omeprazole, 20 mg/kg	Extract, 200 mg/kg body weight	Extract, 400 mg/kg body weight	Extract, 800 mg/kg body weight
RBC ($x10^6/mm^3$)	7.38 ± 0.44^{c}	6.21 ± 0.17^{a}	$6.57\pm0.22^{a,b}$	$6.90\pm0.28^{b,c}$	7.13 ± 0.32^{c}	7.16 ± 0.17^{c}
PCV (%)	45.00 ± 2.00^{c}	39.00 ± 1.00^{a}	41.67 ± 1.53^{b}	$43.33\pm1.56^{b,c}$	44.67 ± 1.53^{c}	44.67 ± 1.16^{c}
Hb (g/dl)	16.00 ± 0.50^{b}	14.47 ± 0.45^{a}	14.30 ± 0.36^{a}	15.40 ± 0.46^{b}	15.73 ± 0.64^{b}	16.27 ± 0.21^{b}
WBC ($x10^3/mm^3$)	8.82 ± 0.29^{a}	11.20 ± 0.80^{b}	10.42 ± 0.48^{b}	9.39 ± 0.50^{a}	9.27 ± 0.26^{a}	9.35 ± 0.78^{a}
PLT ($x10^3/mm^3$)	236.67 ± 7.37^{a}	245.33 ± 6.51^{a}	237.67 ± 5.03^{a}	239.33 ± 8.51^{a}	237.67 ± 1.53^{a}	234.33 ± 6.03^{a}
MCV (fl)	61.04 ± 1.03^{a}	62.77 ± 0.21^{b}	63.42 ± 0.32^{b}	62.80 ± 1.02^{b}	62.67 ± 0.74^{b}	62.41 ± 0.23^{b}
MCH (pg)	21.72 ± 0.64^{a}	23.28 ± 0.16^{c}	21.78 ± 0.73^{a}	$22.32\pm0.44^{a,b}$	$22.07\pm0.15^{a,b}$	$22.73\pm0.25^{b,c}$
MCHC (g/dl)	35.57 ± 0.47^{b}	37.09 ± 0.22^{c}	34.34 ± 1.14^{a}	35.54 ± 0.70^{b}	35.22 ± 0.33^{b}	$36.43\pm0.50^{b,c}$

Values are presented as mean ± standard deviation (n = 3), and values with different letter superscripts are significantly ($P < 0.05$) different across the row.

Effect of liver function marker on ethanol extract of *Dialiumguineense* stem bark

The result of Total Protein showed no significant different ($P < 0.05$) between ulcer control group and omeprazole group and they are significantly lower ($P < 0.05$) when compared to the groups treated with normal control and groups treated with 200mg/kg, 400mg/kg and 800mg/kg body weight of the extract. In Albumin result, the groups treated with omeprazole and 200mg/kg, 400mg/kg and 800mg/kg body weight of the extract and they are significantly higher ($P < 0.05$) than the ulcer group. The ALT and AST and ALP results showed no significant different in the normal control and the groups treated with 200mg/kg, 400mg/kg and 800mg/kg body weight of the extract and are significantly lower ($P < 0.05$) when compared to the ulcer control.

Table 2: Liver function parameters

Treatment groups	Normal control	Ulcer control	Omeprazole, 20 mg/kg	Extract, 200 mg/kg body weight	Extract, 400 mg/kg body weight	Extract, 800 mg/kg body weight
Total protein (g/dl)	6.30 ± 0.28^{b}	5.71 ± 0.22^{a}	5.67 ± 0.33^{a}	$6.02\pm0.10^{a,b}$	6.38 ± 0.21^{b}	6.40 ± 0.44^{b}
Albumin (g/dl)	3.50 ± 0.11^{b}	2.54 ± 0.30^{a}	3.14 ± 0.31^{b}	3.47 ± 0.08^{b}	3.33 ± 0.15^{b}	3.42 ± 0.21^{b}
Globulin (g/dl)	$2.79\pm0.19^{a,b}$	3.17 ± 0.52^{b}	2.54 ± 0.04^{a}	2.55 ± 0.04^{a}	3.05 ± 0.06^{b}	$2.98\pm0.24^{a,b}$
ALT (u/l)	24.67 ± 1.32^{a}	71.00 ± 6.25^{e}	61.33 ± 2.31^{d}	54.00 ± 5.57^{c}	$49.67\pm3.51^{b,c}$	46.33 ± 2.41^{b}
AST (u/l)	35.33 ± 1.16^{a}	85.33 ± 3.22^{e}	69.00 ± 3.61^{d}	54.67 ± 4.16^{c}	$52.00\pm2.00^{b,c}$	46.67 ± 2.89^{b}
ALP (u/l)	$84.33\pm4.51^{a,b}$	92.33 ± 3.51^{b}	82.00 ± 2.00^{a}	83.00 ± 3.00^{a}	78.67 ± 5.03^{a}	78.33 ± 7.37^{a}
Total bilirubin (mg/dl)	0.68 ± 0.07^{a}	0.84 ± 0.05^{b}	0.72 ± 0.04^{a}	$0.74\pm0.03^{a,b}$	0.68 ± 0.09^{a}	0.70 ± 0.07^{a}

Values are presented as mean ± standard deviation (n = 3), and values with different letter superscripts are significantly ($P < 0.05$) different across the row.

DISCUSSION

Amendment of RBC parameters in ulcer patients are related with different mechanisms; including decreased iron absorption secondary to chronic gastritis Al (Mutawa, O.A. et al 2023), iron loss via hemorrhagic gastritis, active bleeding peptic ulcers, deficiency of iron and vitamin B12 secondary to chronic and atrophic gastritis, which might contribute to the alteration of RBC parameters (Mărginean, C.D et al.2022). There is reduction of RBC in the ulcer group when compared to other groups, this could be as a result of hemorrhage in gastric. This result is supported by the works of Saler et al 2014 and Haile, K. and A. Timerga, 2021, that RBC count of animal models is reduced in conditions of peptic ulcer. The RBC count of the groups treated with the Dialiumguineense showed significant increase indicating that the extract ameliorated the effects by reducing the loss through haemorrhage in the gastric.

An increase level of PCV and Hb concentration in the groups treated with the extract showed an improved condition and are also physiological building of the stomach. An elevation of WBC status is credited to inflammation and leucopenia and general haematological abnormalities. This is supported by the work of Obidike et al 2023. Though the decrease in the concentrations as noticed in the groups that co-treated with the extract of Dialiumguineense stem bark could be suggestive of ameliorative abilities, hence setback of the ulcer induced by ethanol.

Penetration into the liver is an occasional complication of peptic ulcer disease and may lead to unusual complications such as abscess formation or upper gastrointestinal hemorrhage. A study by Dore MP et al 2024, showed that the incidence of PUD was nearly 8 times greater in a group of patients with cirrhosis who were infected with H pylori compared with uninfected patients with cirrhosis. Therefore. In this presence study, there are significant increase ($P < 0.05$) in the level of ulcer group of ALT and AST and ALP. When compared to the treated with omeprazole and the ethanol extract of Dialiumguineense. This may be as a result of gastrointestine haemorrhage in the ulcer control group.

CONCLUSION

The result of this study showed that the extract of Dialiumguineense is an effective antiulcer agent and it has the ability to ameliorate the effects of ulcer the on the liver. It also showed that the extract helps to improve the level of some haematological indices such as RBC, Hb and PCV.

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