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Amilioretive Effect of Aqueous Leaves Extract of *Ziziphus mucronata* in Ethanol Induced Gastric Ulcer Model Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author AD designed the study, wrote the protocol and performed the statistical analysis. Author SAZ handle the animals, wrote the first draft of the manuscript and manage the literature searches. Author KA managed the histopathological analysis. Author SR manage the antioxidant Vitamin analyses. Author CAO handle the haematological analysis. All authors read and approved the final manuscript.

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ABSTRACT

Background: Gastric ulcer is a serious problem affecting 10% of the global population. *Ziziphus mucronata* is a plant with many reported health promoting effects. The present study was designed to investigate the therapeutic effect of aqueous leaves extract on ethanol induced gastric ulcer model in rats.
Materials and Methods: The rats weighed 170 – 200 g were divided into four groups: Negative control, Positive control, Extract treated (250 mg/Kg), Omeprazole treated (20 mg/Kg). Standard methods were adopted in the analysis of all parameters.
Results: The results of this study revealed no significant differences (P>0.05) on both biochemical and haematological parameters in the extract treated group with exception of HGB which differ

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significantly ($p<0.05$) when compared to positive control. Ethanol administration resulted in decreased levels in serum antioxidant vitamins A, C and E. This effect has been relieved by the extract administration and omeprazole as well. Significant increased ($P<0.05$) of antioxidant enzyme (SOD) activity was observed in ethanol induced ulcer untreated groups. The results of haematological indices revealed that there is significant decreased ($p<0.05$) in RBC, HGB, and HCT and increased in WBC in ethanol induced ulcer untreated ($p>0.05$) compared to other groups. Histopathological evaluation showed transmural architecture of the stomach with clearly defined mucosa, sub-mucosa and muscularis propria in extract treated compared to positive control which is characterized by moderate-mild discontinuity in the mucosa.

Conclusion: The plant extract possess healing properties against stomach lesions, hence may be used in the management of gastric ulcer.

Keywords: Antioxidants; heamatological indices; mucosa; ulcer; Ziziphus mucronata.

1. INTRODUCTION

Ulcer is one of the high ranking global health challenges affecting approximately10% of the population [1]. It is one of the common defects of the gastric or intestinal walls, clinically expressed as abdominal stress, most often in the upper part of the abdomen and epigastric region. Gastric and duodenum ulcerations are commonly known as peptic ulcer [2], which is characterized with the break off in the continuity of the mucosa of stomach or duodenum as a consequences of some medications, gastric acid and pepsin which gradually erode the line of intestinal mucosa and eventually result to intestinal lesion [3]. Certainly, *Helicobacter pylori* infection is a key factor in the onset of majority of ulcers [4]. Other factors that predispose to the development of ulcer include stress (usually due to Illness), alcohol consumption, tobacco smoking, use of non-steroidal anti-inflammatory drugs (NSAIDs) [5]; [6].

It was reported that ethanol provoke ulcer via perturbations of superficial epithelial cells, particularly the mucosal mast cells, resulting to the release of vasoactive mediators such as histamine among others[7] and reactive oxygen species (ROS), [8] resulting in the erosion of gastric mucosa [9].

Alcohol readily penetrates the gastric mucosa thereby causing damage to the cell and plasma membranes leading to increased intracellular membrane permeability to sodium and water. This results to cell death and erosion of the surface epithelium [10].

In addition, ethanol has been implicated in free radicals generation [11]. Mucosal damage can occur by the generation of exogenous and endogenous free radicals. Oxygen radicals are injurious to the integrity of biological tissues. Lipid peroxidation was seen as the mechanism by which cell membranes are destroyed and leads to intracellular components release, such as lysosomal enzyme [12]. The effect of ethanol on gastric mucosa is linked to extensive mucosal capillaries damage and increased vascular permeability [13].

Ziziphus mucronata wild, commomly known as Cape thorn or Buffalo thorn is a small to medium sized tree, with a spreading canopy belonging to the family Rhamnaceae. It is distributed along the rainfall areas of sub-Saharan Africa including Nigeria [14]. The fruits and leaves powder of *Ziziphus mucronata* have long been applied to treat boil. Root extracts has been orally administered in the treatment of abdominal pain and infertility in women, whereas the root powder is applied on wounds [15].

The plant is used to heal illness like dysentery, swellings, chest pains, tooth ache, eye diseases, swollen and open wounds [16]. It was reported that the roots of *Ziziphus mucronata* is a strong radical scavenging and potent acetylcholine inhibitory activity [17]. In ethno-medicine, the roots, barks and leaves are used to treat boils, swollen glands, wounds, sores and to purify and improve skin complexion [18]. Many researches indicated the pharmacological activities of *Ziziphus mucronata*, especially on gastrointestinal tract and there is a lot of non scientific based evidence on the health promoting potential of the plant. It is therefore a great interest to evaluate the effect of this plant against ulcer.

2. MATERIALS AND METHODS

2.1 Collection of Plant

The leaves of *Ziziphus mucronata* were collected with the help of a traditional herbalist from

Zarewa Town Kano State, Nigeria and identified by an expert in the Department of Biological Sciences, Usmanu Danfodiyo University Sokoto and assigned voucher number: UDUH/ANS/0223. It was then dried under shed and grinded by the use of pestle and mortar. The powdered form was sucked in water for 48hrs (150g in 800ml of distilled water) then filtered. The filtrate was evaporated in a drying cabinet at a temperature of 40 - 45℃. The solid extract obtained was stored in polythene bags till required for the experiment.

2.2 Experimental Design

2.2.1 Experimental Animals

Total of 28 animals ranged from 170 to 200g were used in this research. The animals were allowed to acclimatize to the laboratory for two weeks and fed with grower mesh pellets of Vital Feeds Limited, Jos, and have free access to water.

The experimental rats were randomly grouped into five of seven rats each.

Group I: placed on distilled Water (Negative control)
Group II: Ethanol induced ulcer untreated (Positive control)
Group III: Ethanol induced ulcer treated with 250 mg/Kg of the extract
Group IV: Ethanol induced ulcer treated with 20 mg/Kg Omeprazole

2.2.2 Ulcer Induction

The rats in group (I - IV) were starved for 48 hrs with free access to water to ensure an empty stomach, then the animals in group II, III and IV were orally dosed with 80% ethanol (5 ml/Kg body weight) through the gastric gavage needles and allowed for 24 hrs for the onset of ulcers. Then extract and omeprazole treatments were followed for 28 days.

2.2.3 Sample Preparation

Twenty four (24) hours after the last treatment, the animals were anaesthetized with chloroform vapour then sacrificed under the supervision of a veterinarian. The blood samples were collected and prepared for biochemical investigations. The stomach was harvested, washed with normal saline and stored in neutral buffered formalin for histopathological studies.

2.2.4 Determination of antioxidant enzymes

Catalase (CAT): Determination of catalase activity was achieved by the method described by Beers and Sizer, [19]

Superoxide Dismutase (SOD): The activity of SOD was measured according to the method described by Zhou et al. [20].

Vitamin A: The serum vitamin A concentration was assayed according to the method described by Rutkowski et al. [21].

Vitamin C: The concentration of Vitamin C was evaluated based on the method of Rutkowski et al. [22]

Vitamin E: The serum Vitamin E level was measured based on the method of Rutkowski et al. [23].

2.2.5 Haematological Analysis

Samples were analyzed with a hematology auto analyzer (PCE-210 N; Erma, Inc, Tokyo, Japan), Analyses were performed according to the standard operating manual.

2.3 Histopathological Evaluation

The harvested stomach was fixed in neutral buffered formalin for 24 hrs. The tissue was then fixed in 10% buffered formalin and processed using a tissue processor. The slides were examined by a Histopathologist at the Usmanu Danfodiyo University Teaching Hospital (UDUTH) Sokoto, in the Department of Histopathology, for morphologic changes.

2.4 Data Analysis

Data generated were presented in tabular form and expressed as mean ± Standard Deviation.

The values were tabulated and presented as Mean ± Standard Error of the Mean (SEM). Results were statistically analyzed by one way ANOVA, using Graph pad Instat software version 3.0 Sen Diego USA. Tukey multiple comparison was used to compare the difference between the means. The differences were considered statistically significant at $P<0.05$.

The results of the Histopathological studies were presented in form of photomicrographs.

3. RESULTS

The results of the effect of aqueous leaves extract of *Ziziphus mucronata* on antioxidant vitamins and enzymes were presented in table 1 below. The result demonstrated that vitamin A, C and E were increased significantly ($p<0.05$) in extract treated group compared to other groups with exception of normal control group which appeared similar. SOD activity was significantly increased in omeprazole treated group and significantly decreased ($p<0.05$) in normal control group compared to other groups. On the other hand, no significant different ($p> 0.05$) was observed in the catalase activity across the groups.

3.1 Haematological Indices

The results of the effect of aqueous leaves extract of *Ziziphus mucronata* on Haematological parameters was represented in table 2 below. The results showed certain significant changes ($P<0.05$) in WBC, RBC, HGB HCT and PLT across the groups. On the other hand, non significant change ($P>0.05$) was observed in the level of MCV, MCH and MHCH among the groups.

3.2 Histopathological Analysis Results

The results of histopathological analysis of stomach tissue were presented in Fig. 1-4.

4. DISCUSSION

The study was conducted to investigate the anti-ulcer effect of aqueous leaves extract of *Ziziphus mucronata* on the stomach histology and certain biochemical parameters.

The results of the Biochemical analysis showed certain significant alterations in the levels of vitamin A, C and E. Ethanol administration resulted in decreased levels in serum antioxidant vitamins A, C and E. This effect has been relieved by the extract administration and omeprazole

Table 1. Effect of aqueous leaves extract of *Ziziphus mucronata* on antioxidant enzymes and vitamins of ethanol induced ulcer rats

Parameters	Groups			
	Group I	Group II	Group III	Group IV
Vitamin A (µmol/L)	0.67 ± 0.26^{a}	0.21 ± 0.09^{ab}	1.27 ± 0.29^{ac}	0.45 ± 0.31^{ab}
Vitamin C (µmol/L)	22.29 ± 4.84^{a}	19.73 ± 3.65^{ab}	29.47 ± 7.53^{ac}	26.25 ± 0.44^{abc}
Vitamin E (mg/dL)	819.08 ± 18.31^{a}	331.36 ± 15.81^{b}	795.35 ± 11.26^{a}	557.35 ± 9.72^{c}
Catalase (U/mL)	27.71 ± 6.62	39.71 ± 3.42	25.43 ± 3.44	39.19 ± 2.92
SOD (units/mL)	99.93 ± 14.42^{a}	132.13 ± 13.93^{b}	138.19 ± 7.87^{b}	206.52 ± 62.85^{c}

Values are mean ± standard error of the mean, (n= 6). SOD = superoxide dismutase. The statistical significance is taken at p<0.05. Those values with different superscript in a row are statistically significantly (p<0.05) while values with the same superscript are statistically non significant.

Table 2. Effect of aqueous leaves extract of *Ziziphus mucronata* on Haematological parameters of ethanol induced ulcer rats

Parameters	Groups			
	Group I	Group II	Group III	Group IV
WBC ($x10^3$µL)	6.13 ± 0.17^{a}	10.23 ± 1.72^{b}	7.33 ± 0.73^{a}	10.77 ± 2.83^{b}
RBC ($x10^6$µL)	5.63 ± 0.35^{a}	3.05 ± 0.17^{b}	4.98 ± 0.18^{a}	5.01 ± 0.36^{a}
HGB (g/L)	12.33 ± 0.33^{a}	5.47 ± 0.79^{b}	11.13 ± 2.23^{a}	10.23 ± 0.88^{a}
HCT (%)	36.27 ± 2.05^{a}	16.37 ± 2.19^{b}	30.07 ± 6.43^{a}	29.9 ± 1.59^{a}
MCV (fL)	65.67 ± 1.68	58.20 ± 1.09	61.00 ± 1.26	60.00 ± 1.56
MCH (pg)	21.97 ± 1.39	19.10 ± 0.59	22.73 ± 0.92	20.50 ± 1.19
MCHC (g/dL)	33.33 ± 1.47	28.50 ± 5.26	37.20 ± 1.69	34.10 ± 1.49
PLT ($x10^3$µL)	250.33 ± 55.48^{a}	552.02 ± 59.51^{b}	406.67 ± 33.74^{ab}	309.33 ± 52.18^{a}

Values are mean ± standard error of the mean (n=6). WBC- White blood cells, RBC- Red blood cells, HGB- Haemoglobin, HCT- Haematocrit, MCV- Mean corpuscular volume, MCH- Mean corpuscular haemoglobin, MCHC- Mean corpuscular haemoglobin concentration, PLT- Platelets. Those values with different superscript in a row are statistically significant (p<0.05) while values with the same superscript are statistically non significant.

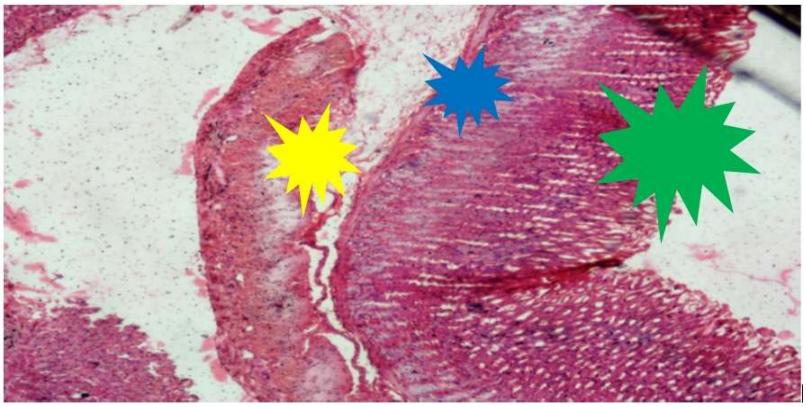

Fig. 1. Negative control: Section of the Stomach showing mucosa (green asterisk), submucosa (blue asterisk) and muscularis propria (yellow asterisk). Hematoxylin & Eosin (H & E) X 40 magnification

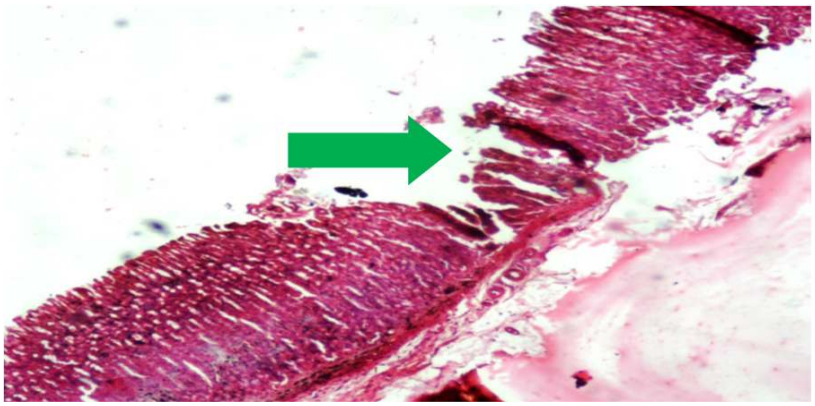

Fig. 2. Positive Control. Section of the Stomach Showing a discontinuity in the mucosa (mild-moderate ulceration) (green arrow). H & E X 40 magnification.

as well. The increased in serum antioxidant vitamins observed in extract treated group might be associated with appreciable content of these vitamin in *Ziziphus* as earlier reported by Sharma et al. [24].

The significant increased ($P<0.05$) of antioxidant enzyme (SOD) activity observed in ethanol induced ulcer untreated groups could be due to system response to stress induced by ethanol. During stressful condition, the SOD activity increased in order to counter the damaging effect of oxygen radicals [25]. It was also observed that neither the extract nor the omeprazole have normalized SOD activity.

The results of haematological indices revealed that there is significant decreased ($p<0.05$) in RBC, HGB, and HCT in ethanol induced ulcer untreated compared to other groups. However, MCV, MCH an MCHC remained statistically similar ($P>0.05$) across the groups. The decreases of these parameters may be due to increase RBC haemolysis resulting from increase erythrocyte fragility and impairment of the antioxidant defense mechanisms [26]. Thus, the decreased of RBC, HGB and HCT in ethanol induced ulcer untreated rats might be a reflection of oxidative stress effects of ethanol on red cell membrane or due to bone marrow suppressive effect of ethanol [27]. These effects have been countered and the level of this parameters was improved toward normalcy due to both extract and omeprazole administration. The increase in total WBC observed in ethanol induced untreated group and omeprazole treated group could be link to the system immune responses occurring in the lining of the digestive tract. Moreover, the increased level of PLT in the ethanol induced ulcer untreated group is associated to increased

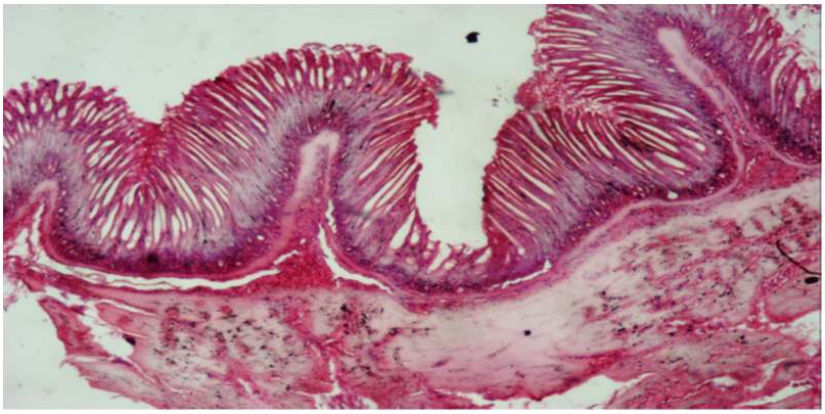

Fig. 3. Extract treated. section of the stomach showing preserved transmural Architecture. H & E X 40 magnification

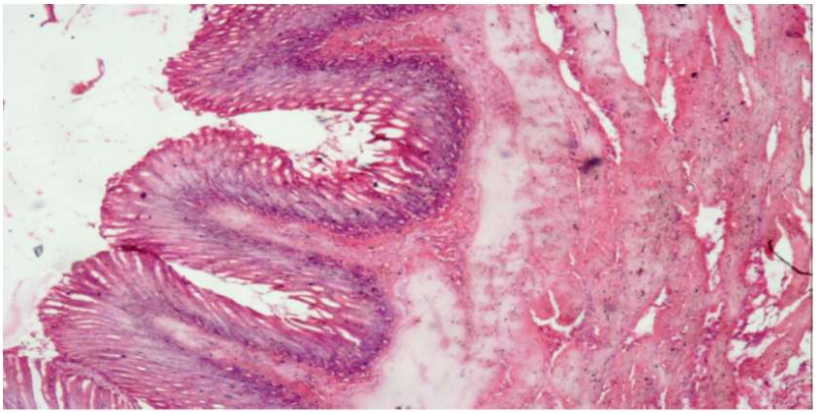

Fig. 4. Omeprazole treated control. section of the stomach showing preserved transmural architecture. H & E X 40 magnification

production of the blood clotting factors to prevent excessive loss of blood, a consequence of stomach lesion caused by ethanol [28]. These parameters were improved in the groups treated with extract and omeprazole. However, no significant change was observed in omeprazole treated group compared to ethanol induced ulcer untreated group. Similarly, Platelets counts remain statistically similar ($P>0.05$) in the comparison made between extract treated group and ethanol induced untreated group, although reduction by 26% due to extract administration was observed.

It was suggested that the antioxidant potential of the extract might be responsible for preventing the destabilizing effect of ethanol on erythrocytes membrane. Aqueous extract of *Z. mucronata* may have a positive impact in the hematopoietic system, which seems to have manifested in the levels of RBC, HGB and HCT. Perhaps, due to its iron content. Niamat et al. [29]. The observed increase in the haematological parameters may also be due to the extract ability to improve bone marrow functions, a major site for erythropoiesis [30].

It was reported that oral administration of ethanol causes the impairment of gastric defensive factors such as mucus and mucosa circulation and, in this way; it induces the necrotic lesions of the gastric mucosa. Typical histological findings of ethanol induced ulcerations are linear hemorrhagic lesions, extensive submucosal edema, mucosal friability, inflammatory cells infiltration, and epithelial cell loss in the stomach [31].

In this study, gastric ulceration was induced by the administration of ethanol to the experimental animals. The lesion was clearly observed and characterized by a mild-moderate discontinuity in

the mucosa. This finding is consistent with ulceration diagnosis (mild-moderate peptic ulceration). However, the administration of aqueous leaves extract of *Ziziphus mucronata* showed transmural architecture of the stomach with clearly defined mucosa, submucosa and muscularis propria. The results have evidently indicated that aqueous leaves extract of *Z. mucronata* possess potential antiulcer agent and as such can have a good curative agent against gastric ulcer.

5. CONCLUSION

The present study revealed that *Ziziphus mucronata* had improved antioxidant vitamins levels, and heamatological indices towards normal as well as preserving mucosal tissues in the experimental animal. These lead to the suggestion that the plant extract may possess healing effects on ethanol induced model ulcer in rats. Hence the plant can be employed as alternative source of drug against ulcer.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by Usmanu Danfodiyo University ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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