

Therapeutic Potentials of Desert Date (*Balanites aegyptiaca*) Aqueous Fruit Pulp Extract on Ethanol Induced Gastric Ulcer in Albino Wistar Rats

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ABSTRACT

Background: Gastric ulcer results from a disruption of the mucous membrane of the stomach and it affects about 10% of the global population. *Balanites aegyptiaca* Linn is a common desert plant belonging to the family Balanitaceae. **Aim:** The study aimed to evaluate the therapeutic potentials of aqueous extract of *Balanites aegyptiaca* fruit pulp on an ethanol-induced gastric ulcer rat model. **Methodology:** The Wistar albino rats were randomly divided into six groups of five rats each, group I was the normal control group and were allowed free access to water and feed *ad libitum*, group II-VI fasted for 12 hours before administration of 5ml/kg absolute ethanol (95%) to induce gastric ulceration. Group II serve as negative control and was administered normal saline and allowed free access to water and feed *ad libitum*. The experimental groups, III-V were administered 100mg/kg, 150mg/kg, 200mg/kg of the extract for seven days respectively while group VI received 20mg/kg omeprazole for seven days. The rats were euthanized and the stomach was harvested and scored to determine the ulcer score, processed, and stained with Hematoxylin and Eosin. **Result:** The acute toxicity study showed that the extract was not toxic and have an $LD_{50} > 5,000$ mg/kg, the following phytochemicals were shown to be present: Tannins, Saponins, Alkaloid, Steroids, and Flavonoid. The mean ulcer score significantly reduced to 1.50, 1.00, and 1.30 in the medium, high dose, and omeprazole groups respectively when compared to the negative control group (8.00) as well as reversal of ulceration across the treated groups was observed in a dose-dependent manner as the epithelial lining of the stomach was observed to regenerate gradually. **Conclusion:** The study showed that the aqueous extract of *Balanites aegyptiaca* has therapeutic properties against ethanol-induced gastric ulceration by reducing ulcer index and promoting gastric mucosal regeneration.

Keywords: Gastric ulcer, ulcer index, *Balanites aegyptiaca*, Ethanol and Ulcer Model,



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INTRODUCTION

A gastric ulcer is a mucosal lesion of the stomach. The patient may have a normal acid secretory rate, it develops when there is disruption of the mucosal lining of the stomach and the balance between acid-pepsin secretion is disrupted. A gastric ulcer may penetrate beyond the mucosa of the stomach. It is histologically similar to duodenal ulceration and are both referred to as peptic ulcers^[1]. The Centre for Disease Control and Prevention (CDCP) reported that ulcers occur due to the damaging effect of digestive juice on the protective lining of the stomach. It may also be a result of activities of *Helicobacter Pylori* which is a bacteria present in the mucosal layer of the stomach and researchers have associated the bacteria with 90% of duodenal ulcers and 80% of gastric ulcers, in southwestern Nigeria almost 100% of duodenal ulcer and 82% of gastric ulcer patients are *Helicobacter pylori*-positive^[19]. As a potential risk factor of gastric ulcer development, ethanol is capable of penetrating the gastric mucosa resulting to the solubilization of the protective mucous and then exposing the mucosa to the proteolytic and hydrolytic actions of hydrochloric acid and pepsin, which will subsequently lead to membrane injury. Ethanol can stimulate acid secretion, reduce blood flow via disruption of vascular endothelium^[10]. The search for novel treatment of ulceration has resulted in experiments with several herbs known to have antioxidant potentials, such as *Camellia Sinensis*, *Uncaria tomentosa*, *Ganoderma lucidum*, *Olea europaea* leaves, and mastic extract among many others, to treat ulceration of the gastrointestinal tract^[6]. Some of the treatments in use for the management of gastric ulceration are not without complications (bleeding, infection, and scar tissue)

as reported by Bhowmik et al.,^[5] *Balanites aegyptiaca* is a common desert plant belonging to the family *Balanitaceae* which grows widely in semi-arid and arid regions of the Middle East, South Asia, and Africa. *Balanites aegyptiaca* has been used to treat different medical conditions. Khare demonstrated the spermicidal activity of aqueous extract of *Balanites aegyptiaca* fruit pulp and found out that 4% of sperm cells become sluggish upon contact with the fruit pulp extract and immobilized after 30 seconds of administration, though the effect observed was dose-dependent^[12]. Furthermore, testicular dysfunction was also observed in dogs following its administration, and this effect was attributed to the presence of treatment-induced hyperglycemia^[12]. In addition, Eze et al.^[8] reported that oil from the seeds of *Balanites aegyptiaca* moderately reduce ulcer mean severity score in guinea pigs while Ashaal et al.^[4] reported that the oil had an anti-carcinogenic effect against liver, lung, and human brain carcinoma cells, besides having anti-mutagenic properties against *Fasciola gigantica* induced mutagenicity^[4]. In an experiment using mice, it was shown that *Balanites aegyptiaca* extract exhibited antioxidant properties against o-adriamycin induced cardiotoxicity that led to a rise in serum levels of lactate dehydrogenase, creatine phosphokinase, glutamine oxaloacetate transaminase, glutamate pyruvate transaminase, lipid peroxide plasma catalyst, erythrocyte lysate superoxide dismutase, glutathione peroxide and total nitric acid in the heart tissue of mice^[15] The fruit pulp of *Balanites aegyptiaca* has been shown to have pain healing and antioxidant properties, it is also claimed by traditional medical practitioners to have anti-ulcer properties. However, there is no scientifically documented evidence on it. It is

against this background that this study seeks to determine the therapeutic potentials against gastric ulcers using ulcer index and histopathological study of the stomach.

METHODOLOGY

Experimental design

The study was approved by the Department of Human Anatomy university of Maiduguri and the rats used were handled following the guidelines for the care and use of laboratory animals by the National Research Council [18]. Ethanol induced gastric ulcer in Albino Wistar rat models were used because they resemble ulcer in humans and is independent of gastric acid secretion. The Albino Wistar rats used for the experiment were procured from the department of biochemistry university of Maiduguri, acclimatized for one week and were randomly grouped into six groups of five rats each. Group I served as normal control and were allowed access to water and feed *ad libitum*. Group II-VI were fasted for 12 hours before administration 5ml/kg absolute ethanol (95%) to induce gastric ulcers. Group II served as negative control and was given distilled water daily for seven days to mimic the intubation of extract to experimental groups (III, IV, V, and VI), and feed *ad libitum*. Groups III, IV, and V were administered 100mg/kg, 150mg/kg, and 200mg/kg aqueous extract of *Balanites aegyptiaca* fruit pulp respectively while the positive control group (group VI) were administered 20mg/kg omeprazole for seven days. On the eighth day of the experiment, the rats were euthanized through cervical dislocation and the stomach of each rat was harvested for histopathological observation. The stomach tissue harvested was dissected, washed, and scored for ulcer, the ulcer score was determined based on the severity of

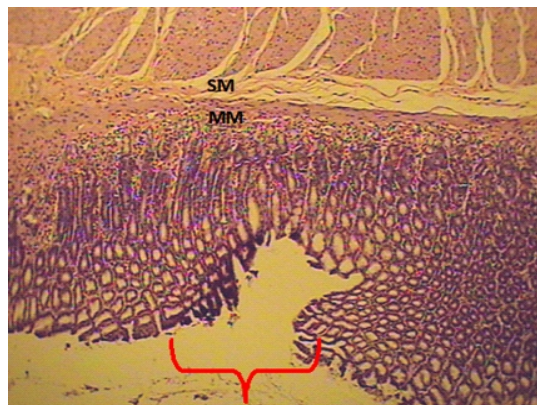
mucosal ulceration. A system of scoring was adopted from Saheed *et al.* [21] and used to grade severity and intensity as follows: No lesion = 0; small round haemorrhagic lesion/vascular congestion = 1; one or two lesions = 2; severe lesions = 3; very severe lesions = 4; mucosa full of lesions = 5. The mean score will be calculated and expressed as Ulcer index (UI) [21]. The stomach tissues harvested from the rats were processed using routine histological technique and stained with Hematoxylin and Eosin (H&E) as described by Young *et al.* [29]. The slide was viewed by the researchers and a pathologist. Numerical data obtained from the study were analysed using InStat software application (Graph Pad 3.5). One-way analysis of Variance (ANOVA) was used to determine the differences between group. The data are expressed as mean \pm SD. p value <0.05 was considered statistically significant.

MATERIALS

Balanites aegyptiaca fruit was purchased from Monday Market Maiduguri, the fruit epicarp was removed manually to expose the fruit mesocarp which was then soaked for 72 hours in 100mls of distilled water at room temperature. The mixture obtained was filtered to separate the solution of the fruit mesocarp from the nuts using a filter and a stirrer. The filtrate was placed in the oven at 40-45°C to evaporate its water content leaving behind the concentrated aqueous solution of *Balanites aegyptiaca* fruit pulp with a PH of 5.1, the extract was stored in a refrigerator. The aqueous extract of *Balanites aegyptiaca* fruit pulp was qualitatively screened for the presence of tannins, saponins, alkaloids, steroids, glycoside, and flavonoid. The acute toxicity study was done according to the method described by Lorke [14], the LD₅₀ was calculated using the formulae:

RESULTS

The phytochemical screening of aqueous extract of *Balanites aegyptiaca* fruit pulp extract revealed the presence of the Tannins, Saponins, steroids, Alkaloid, and Flavonoids while the acute toxicity studies showed that the extract had no toxic effect in Albino Wistar rats at 5,000mg/kg.



B

Table. 1. Therapeutic effect of *Balanites aegyptiaca* fruit extract on ulcer index

N=5	
Groups	Ulcer Index
Control	0.00 ± 0.00
0mg/kg extract	8.00 ± 3.90
100mg/kg extract	5.00 ± 2.60
150mg/kg extract	1.40 ± 0.90**
200mg/kg extract	1.00 ± 0.00**
20mg/kg Omeprazole	1.30 ± 0.50**

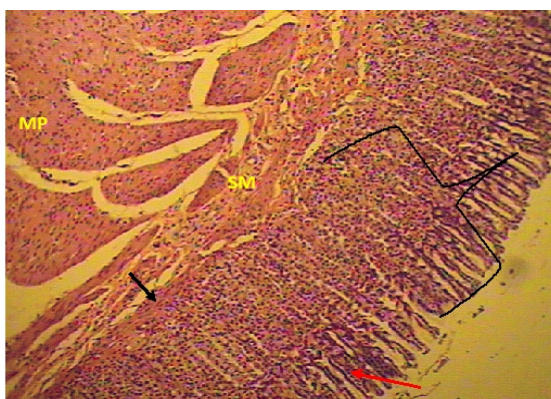
Values are expressed as mean ± standard deviation

Ulcer index expressed and mean ulcer score

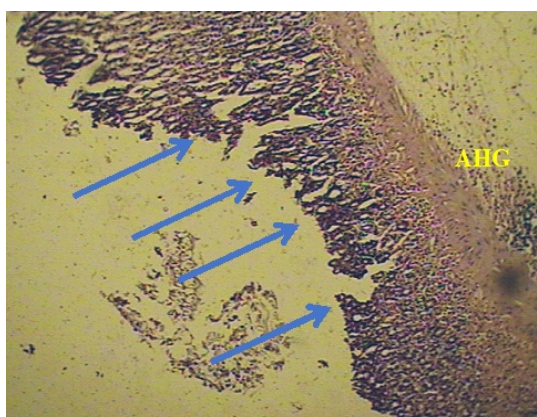
**= statistically extremely significant P<0.01



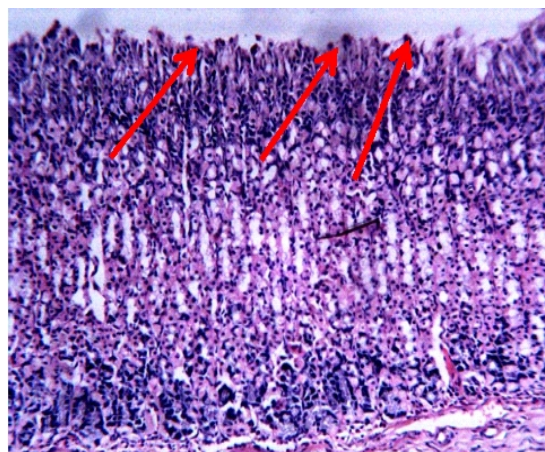
C



A



D

**E****F**

A: Photomicrograph of normal rat stomach showing normal structures stained with H&E x100 Magnification. Submucosa (SM), Muscularis Propria (MP), Muscularis mucosae (MM), tubular mucus glands (black brace) and foveola (red arrow), B: Photomicrograph of ulcerated rat stomach administered normal saline showing focal ulceration of epithelium (red brace) with normal Muscularis mucosae (MM) and submucosa (SM) H&E x100 magnification, C: Photomicrograph of ulcerated rat stomach treated with 100mg/kg aqueous *Balanites aegyptiaca* fruit pulp extract showing focal mild ulceration of the simple tubular mucous secreting glands (red arrows) with normal Muscularis mucosae (MM) H&E x100 magnification, D: Photomicrograph of ulcerated rat stomach treated with 150mg/kg aqueous extract of *Balanites aegyptiaca* fruit pulp extract showing ulceration undergoing regeneration (blue arrows) and inflammatory cells (AHG) H&E x100 magnification, E: Photomicrograph of ulcerated rat stomach treated with 200mg/kg aqueous *Balanites aegyptiaca* fruit pulp extract showing mild disruption of surface epithelium (arrows) H&E x100 magnification, F: Photomicrograph of ulcerated rat stomach treated with 200mg/kg aqueous *Balanites aegyptiaca* fruit pulp extract showing mild disruption of surface epithelium (arrows) H&E x100 magnification.

DISCUSSION

The qualitative phytochemical screening confirmed the presence of Tannins, Saponins, alkaloids, Steroid, and Flavonoid in the extract, the findings were consistent with the report by Usman *et al.*^[26]. However, Mhya *et al.*^[16] reported that the extract has phenol, Saponins, Flavonoid, and Tannin. Aqueous *Balanites aegyptiaca* fruit pulp

reduced ulcer index. In the present study rats administered aqueous extract of *Balanites aegyptiaca* fruit pulp showed reversal of ulceration at medium and high dose and also in Omeprazole treated group, Nahla *et al.*^[17] showed that *Cibotium barometz* leave extract protects against a gastric ulcer in rats. Omeprazole a proton pump inhibitor

extract was found by Salihu *et al.*,^[22] to contain alkaloids, Steroids, Flavonoids, and glycoside. In addition to the findings from the present study, Tesfaye reported the presence of Coumarins and Diosgenin^[24]. The phytochemical studies of *Balanites aegyptiaca* by Alhassan *et al.*^[3] also confirmed the presence of tannins, saponins, alkaloids, steroids, flavonoids, and in addition the presence of glycoside. The acute toxicity studies produced no sign of acute toxicity and mortality in all the study groups. Thus, LD₅₀ was 5000mg/kg, a similar result was obtained by Ajaji and Folorunso^[2]. Alhasan *et al.*^[3] reported that *Balanites aegyptiaca* is non-toxic and have many physiological activities hence they are widely used in medicine. This study demonstrates that ethanol-induced gastric ulceration, a similar result has been reported by several researchers^{[17]; [20]; [27]}. Histological observation demonstrates damage to gastric mucosa in the negative control group (0mg/kg extract) and the treated groups, furthermore, the experimental groups have inflammatory cells as shown in figure D, otherwise, rats treated with aqueous *Balanites aegyptiaca* fruit pulp extract showed a dose-dependent reversal of mucosal ulceration and rats treated with Omeprazole show reversal of gastric mucosal similar to the extract-treated groups. The ulcer index decreased in the experimental groups, the ulcer index decreased from 8 in the negative control group to 1 and 1.3 in the high dose (200mg/kg extract) and Omeprazole groups (20mg/kg omeprazole) respectively. Plant extracts such as *Aspilia Africana* and *Anthocesta Vogeli* have been shown to reduce ulcer index in rat models^[23], a similar result was obtained in the study of the effect of *Entandrophagma* on a gastric and duodenal ulcer in rat models by John *et al.*,^[25]. Wei-Ping *et al.*^[28] showed that *Salvia miltiorrhizah* significantly

inhibits acid secretion into gastric mucosa. The anti-ulcer properties of the extract could be due to its antioxidant properties as reported by Gadzama *et al.*^[9].

CONCLUSION

The aqueous extract of *Balanites aegyptiaca* fruit pulp was shown to be non-toxic and the phytochemical screening of the extract confirmed the presence of Tannins, Saponin, Alkaloid, steroids, and Flavonoids. The study has also demonstrated that seven days administration of the extract has therapeutic potential against ethanol-induced gastric ulceration by promoting gastric mucosa regeneration and communities using it to control symptoms of gastric ulceration should do so with caution as further studies is needed to know if it affect other biological system.

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