ISSN: 2782-7550 (Print)
ISSN: 2782-7542 (Online)

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ANNALS OF BASIC AND MEDICAL SCIENCES

A Scientific Peer Reviewed Publication of The Faculties of Basic Medical
and Basic Clinical Sciences, Usmanu Danfodiyo University Sokoto, Nigeria

Studies on the Antiulcer Activity of the Chloroform Fraction of *Balanites aegyptiaca* Stem Bark Extract

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Abstract

Background: *Balanites aegyptiaca* L. Delile (Zygophyllaceae) is a perennial tree found mostly in the desert environments. Decoctions of its stem barks are used traditionally in northern Nigeria to treat wounds, ulcers and stomach ailments. Other ethnomedicinal uses include: treatment of malaria, pain and fever. The aim of this study was to evaluate the antiulcer activity of the chloroform stem bark extract of *Balanites aegyptiaca* in Wistar rats.

Materials and methods: Acute toxicity study on the chloroform fraction of the stem bark extract of *Balanites aegyptiaca* was carried out using the OECD 425 guidelines at a limit dose of 3000 mg/kg. Phytochemical analysis of the fraction was carried out to detect the presence of alkaloids, tannins, flavonoids, steroids, carbohydrates, saponins and glycosides using standard procedures. The antiulcer activity of the fraction was evaluated in ethanol and indomethacin-induced ulcer models in rats. Rats were allotted into five groups (n=5). Group 1 received 10% Tween 20 (1 ml/kg), group 2 received standard drug, omeprazole (20 mg/kg), and groups 3-5 were the extract groups and received 125, 250 and 500 mg/kg, p.o. respectively of the chloroform fraction of *Balanites aegyptiaca*. The mean ulcer indices were assessed and the percentage ulcer inhibition calculated.

Results: At the limit dose of 3000 mg/kg the fraction of *Balanites aegyptiaca* did not produce any mortality or observable signs of toxicity in the rats. The phytochemical analysis showed the presence of flavonoids, saponins and steroids. The fraction at the doses of 250 and 500 mg/kg caused a significant (p<0.05) reduction in mean ulcer indices in the ethanol and indomethacin-induced ulcer models when compared to the control.

Conclusion: Our study showed that the chloroform fraction of the stem bark extract of *Balanites aegyptiaca* possesses antiulcer activities. Further studies are needed to isolate the active constituents.

Keywords: *Balanites aegyptiaca*; Chloroform fraction; Ethanol-induced ulcer; Indomethacin-induced ulcer; Acute toxicity

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Introduction

Peptic ulcer disease (PUD) arises due to an imbalance between defensive and destructive factors in the stomach (1). Some of the mucosal destructive factors include: hydrochloric acid, pepsin and non-steroidal anti-inflammatory drugs (NSAID) while mucin secretion, prostaglandins, mucosal blood flow amongst others are the defensive factors (2). When the balance is affected and the destructive factors outweigh the defensive factors, ulcer may result. Peptic ulcer disease affects a large proportion of the world population (3) leading to increase in morbidity and health care costs.

The use of acid-reducing pharmacological agents and *H. pylori* eradication regimes are the mainstay of the present-day treatment. Though these therapeutic measures are used, high incidence of relapse, side effects and tolerance have been reported (4). These drawbacks can be overcome with traditional medicines (5).

Traditional medicines form the bases of many pharmacological agents used in modern medicine and are continually being used in the developing countries of the world (6). For many centuries, these traditional medicines have been used for treating several ailments, including ulcer. Some of these plants with antiulcer activities include *Azadirachta indica* (7), *Papaya carica* (8), *Ficus exasperate* (9) and *Balanites aegyptiaca* (10).

Balanites aegyptiaca L. Delile (Zygophyllaceae), commonly called 'Desert date', is a perennial tree found mainly in the desert areas. Decoctions of the stem bark are used as medicine to treat stomach ulcers (11). Previous scientific research had reported the antiulcer activity of the aqueous extract of the plant (10). Since peptic ulcer disease is one of the serious gastrointestinal diseases in the world with major effect on morbidity, it is important to identify cheaper effective medicines that will cure the disease without much side effects. Our previous study on the aqueous extract had verified the claims of previous reports on the knowledge of native traditional medicine practitioners. This experiment further investigated this medicinal plant by evaluating the anti-ulcer effect of its chloroform (a nonpolar solvent) fraction.

Materials and methods

Plant material and fractionation

The stem bark of *Balanites aegyptiaca* was collected from Wamakko local government area in Sokoto state, Nigeria. It was identified and authenticated by Alhaji Umar Mohammed

of the Department of Botany, Usmanu Danfodiyo University, Sokoto (UDUS). Voucher specimen (004B) of the plant was deposited at the herbarium of the same department. The stem bark was air-dried, pulverized using pestle and mortar, and extracted with distilled water using Soxhlet apparatus. A 100 g of aqueous extract of *Balanites aegyptiaca* was dissolved in distilled water and poured into 1 L separating funnel and was exhaustively extracted by consecutive liquid/liquid partition with hexane (500 ml) and chloroform (500 ml) using a separating funnel. The hexane and chloroform fractions were evaporated to dryness to obtain hexane and chloroform fractions (12). The chloroform fraction was used for the study. The percentage yield was calculated using this expression;

$$\% \text{ yield} = W_2/W_1 \times 100$$

W_2 = weight of the extract in grams

W_1 = weight of plant material in grams

Experimental Animals

Male Wistar rats (150–200 g) were obtained from the animal house of the Department of Pharmacology and Toxicology, Usmanu Danfodiyo University, Sokoto. The animals were kept in well-constructed cages and allowed to acclimatise for 2 weeks with free access to standard commercial chow and drinking water before the commencement of the study. Housing conditions were maintained at 25 °C. The experimental protocol was according to the established public health guidelines in guide for care and use of laboratory animals (13).

Phytochemical screening of the chloroform fraction of *Balanites aegyptiaca*

Phytochemical screening of the chloroform fraction of *Balanites aegyptiaca* was conducted to determine the presence of alkaloids, tannins, flavonoid, saponins, carbohydrates, steroids and glycosides using standard procedures (14).

Acute toxicity study

The oral acute toxicity testing of the chloroform fraction of *Balanites aegyptiaca* was carried out using the 'Up-and- Down' method of acute toxicity testing in rats at a single dose of 3000 mg/kg in accordance with the Organization for Economic Development (OECD) 425 guidelines (15). In brief, five female rats were used for the study. An animal was picked at a time, weighed and dosed orally with the fraction dissolved in 10% tween 20. Each animal was observed after dosing for behavioural signs of toxicity and afterwards monitored for 14 days for possible long-term lethal outcome.

Antiulcer evaluation

Ethanol-induced gastric ulcer model

Rats were randomly allotted into 5 groups (n=5). They

were fasted for 24 hours but allowed free access to water for up to 2 hours before the commencement of the experiment (16). Group 1 received 10% tween 20 (1 ml/kg) and served as the normal control while the group 2 was treated with standard drug omeprazole (20 mg/kg). Groups 3-5 were the extract groups and received 125, 250 and 500 mg/kg of the chloroform fraction of *Balanites aegyptiaca* respectively. An hour later, oral gastric lesions were induced with absolute ethanol at 8 ml/kg p.o. After two hours, the rats were sacrificed by cervical dislocation and their stomachs excised and opened along the greater curvature. The ulcer index was determined (17).

Indomethacin-induced gastric model

Rats were randomly allotted into five groups of five animals each. They were fasted as before. Groups 1 and 2 were pre-treated orally with 10% tween 20 (1 ml/kg) and standard drug (omeprazole, 20 mg/kg) respectively. Groups 3-5 were pre-treated with 125, 250 and 500 mg/kg respectively of the chloroform fraction. An hour later, oral gastric lesions were induced with the administration of 100 mg/kg of indomethacin orally to all the groups (18). After four hours, the animals were sacrificed by cervical dislocation and their stomachs excised and opened as before. The ulcer indices were calculated as before.

Measurement of ulcer index

The excised stomachs were cut along the greater curvature and the mucosa were rinsed with cold saline to remove blood contaminants. The ulcerated surface in each stomach was measured with a transparent millimetre scale rule (19). The total lengths of all lesions for each stomach were designated as the ulcer index (UI) and their percentage inhibition calculated. Inhibition percentage was calculated using the expression;

$$\% \text{ Inhibition} = (UIC - UIT)/UIC \times 100$$

Where UIC is Ulcer Index in the control; UIT is ulcer index in the test rats (20)

Data analysis

The results were expressed as Mean $\pm$ SD. Data was analysed using a one-way analysis of variance (ANOVA) followed by Dunnett's t-test for multiple comparisons. GraphPad Prism 6 software was used for statistical calculations. The significant difference was accepted at $p < 0.05$.

Results

Chloroform fraction of *Balanites aegyptiaca* yield

The percentage yield of the chloroform fraction of *Balanites aegyptiaca* was 5.15% w/w.

Phytochemical studies

The fraction showed the presence of flavonoid, saponin and steroid (Table 1).

Table 1. Phytochemical screening of chloroform fraction of *Balanites aegyptiaca*

S/N	Phytochemical constituents	Chloroform fraction
1.	Alkaloids	-
2.	Tannins	-
3.	Flavonoids	+
4.	Saponins	+
5.	Carbohydrate	-
6.	Steroids	+
7.	Glycosides	-

+ Present; - absent

Acute toxicity test

The acute toxicity test using the Up and Down method at an oral limit doses of 3000 mg/kg of the chloroform fraction of *Balanites aegyptiaca* did not produce any sign of toxicity and no mortality occurred within 48 hours after administration and after 14 days of long term observation.

Antiulcer activity

Effect of chloroform fraction of *Balanites aegyptiaca* in Ethanol-induced ulcer model

The chloroform fraction at the doses of 250 and 500 mg/kg significantly ($p < 0.05$) reduced the mean ulcer indices in the rats (Table 2). The percentage inhibition was much lower than that of the standard drug group (Figure 1).

Figure 1: Effect of chloroform fraction of *Balanites aegyptiaca* (CFBA) on percentage ulcer inhibition in ethanol-induced ulcers

Table 2.

Treatment	Dose (mg/kg)	Ulcer index
10% Tween 20	1 ml/kg	10.15 ± 1.25
CFBA	125	8.20 ± 0.54
	250	6.40 ± 0.89*
	500	6.88 ± 0.43*
Omeprazole	20	3.50 ± 0.59**

CFBA = Chloroform fraction of *Balanites aegyptiaca*; Results expressed as mean ± SD, n=5; * $p < 0.05$, ** $p < 0.01$

Effect of chloroform fraction of *Balanites aegyptiaca* in Indomethacin-induced ulcer model

The chloroform fraction produced a significant ($p < 0.05$) reduction in the mean ulcer index at 500 mg/kg when compared to the 10% Tween 20 control group (Table 3). The percentage inhibition produced by 125 and 250 mg/kg were 10% and 6% respectively (Figure 2).

Table 3.

Treatment	Dose (mg/kg)	Ulcer index
10% Tween 20	1 ml/kg	2.45 ± 0.45
CFBA	125	2.20 ± 0.66
	250	2.30 ± 0.79
	500	1.63 ± 0.21*
Omeprazole	20	0.98 ± 0.08**

CFBA = Chloroform fraction of *Balanites aegyptiaca*; Results expressed as mean ± SD, n=5; * $p < 0.05$, ** $p < 0.01$

Figure 2: Effect of chloroform fraction of *Balanites aegyptiaca* (CFBA) on percentage ulcer inhibition in indomethacin-induced ulcers

Discussion

The study evaluated the antiulcer activity of the chloroform fraction of *Balanites aegyptiaca* in Wistar rats using the ethanol and indomethacin-induced ulcer model. The data obtained showed that the fraction possessed antiulcer activity.

To study the cytoprotective effect of the fraction, ethanol-induced gastric ulcer was employed. Ethanol-induced ulcer formation occurs probably due to stasis in gastric blood flow which contributes to the development of the haemorrhage and necrotic aspects of tissue injury (21). It produces mucosal damage by direct necrotizing action which in turn reduces the defensive factors, secretion of bicarbonate and production of mucus (22) leading to gastric mucosal injury. The fraction inhibited the ulcer index in the rats. This is consistent with other antiulcer studies using chloroform fractions (23). This suggests that the chloroform fraction possesses gastroprotective activity.

Indomethacin induces ulcer formation by inhibiting the action of cyclo-oxygenase (COX-1) and (COX-2) activities leading to inhibition of prostaglandins synthase action and direct cytotoxic effect on the

epithelium (24). Secretion of bicarbonate and mucus are stimulated by prostaglandins to maintain the blood flow of the mucosa and regulate the mucosal cell renewal. The inhibition of cyclooxygenase pathway increases the level of leukotrienes in the gastric mucosa and also potentiates the gastric acid secretion effects of histamine (25). Prostaglandin E_2 produced by the gastric mucosa, inhibits secretion of hydrochloric acid and stimulates secretion of mucus and bicarbonate conferring cytoprotective effect on the mucosal layer. The impairment of mucosal defence by indomethacin allows gastric acid to elicit direct erosion of mucosal layer. The fraction prevented this condition only at the higher dose. This suggests that the activity of the fraction may be through prostaglandin-mediated pathway. However, from the data obtained, the fraction could be acting more through prevention of stasis in gastric blood flow than through prostaglandin pathway.

The phytochemical constituents of the chloroform fraction of *Balanites aegyptiaca* were flavonoids, saponins and steroids. Plants that contain flavonoids have been found to be effective in preventing the gastrointestinal tract from ulcerative and erosive lesions mainly because of their antioxidant properties (26). Flavonoids act by increasing the mucosal prostaglandin content, decreasing histamine secretion from mast cells, inhibiting histidine decarboxylase and by the inhibition of *Helicobacter pylori* growth. The presence of these phytochemical components may also contribute to its protective effect by maintaining an effective microvascular supply of gastric mucosa. Saponins protect the stomach mucosa from acid by selectively inhibiting prostaglandin F_2 , which causes vasoconstriction of mucosal blood vessels (27). Plants rich in steroids have also been reported as antiulcer agent (28). This suggests that the antiulcer activity of the fraction may be due to the presence of these phytochemicals.

The oral acute toxicity (LD_{50}) of the chloroform fraction was found to be greater than 3000 mg/kg, this implies that the fraction may be considered to have a high degree of relative safety, devoid of toxicity (29). However, further studies are required to determine its toxic effects due to repeated and chronic administrations.

Conclusion

The chloroform fraction of *Balanites aegyptiaca* protected rats against ulcers induced by ethanol and indomethacin and this may be attributed to the presence of flavonoids, saponins and steroids in the fraction. The LD_{50} of the fraction may be greater than 3000 mg/kg. Further studies are needed to isolate the active principle in the fraction.

Conflict of interest

None declared

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