Journal of Acute Disease

journal homepage: www.jadweb.org

Gastro-protective effect of *Ziziphus abyssinica* root extracts in ethanol-induced acute ulcer in Wistar rats

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ARTICLE INFO

ABSTRACT

Objective: To evaluate the gastro-protective effect of the aqueous, methanol and hexane root extracts of *Ziziphus abyssinica* on ethanol induced gastric ulcer in Wistar rats as models.
Methods: Seventy rats were divided into seven groups of ten rats each: control group, ulcer control group, standard control group (cimetidine 100 mg/kg body weight), aqueous, methanol, ethyl acetate and hexane extract groups. They were administered the extracts at 300 and 600 mg/kg for two weeks before ulcer was induced, and the protective effect of all extracts determined.
Results: Histological changes in gastric tissue were evaluated. Pretreatment with *Ziziphus abyssinica* extracts showed significant ($P < 0.05$) gastro-protective effect with aqueous extract having the highest gastro-protective effect (95% and 93% at 300 and 600 mg/kg extract respectively). Methanol extract had 66.6% and 65.5% protection at 300 and 600 mg/kg respectively. The percentage protection in ethyl acetate extract treated group was 51% and 45% respectively. The group treated with hexane had 23.8% and 28.6% protection at 300 and 600 mg/kg, respectively. Histological study showed that pretreatment with aqueous extract resulted in the preservation of the functional cyto-architecture of the entire mucosa with little pathological changes, compared to other extracts.
Conclusions: The results of this study indicated that aqueous extract is effective against induced gastric ulcer.

1. Introduction

Peptic ulcer is one of the most important causes of morbidity and mortality in both industrialized and non-industrialized countries[1] with symptoms such as epigastric pain, fullness, gas and bloating. It is a benign lesion occurring at a site where mucosal epithelium is exposed to acid and pepsin. Ulcer healing requires angiogenesis in granulation tissue at the base of ulcer, together with re-epithelization starting from ulcer margins and subsequent reestablishment of glandular architecture.The prevention of its pathogenesis and recurrence is the goal of many experimental studies in the present era[2]. A number of products used for the treatment of gastric ulcers, amonsts which are H2-blockers, M1-blockers, proton pump inhibitors, sucralfate and carbenoxolone. Although these drugs brought about remarkable changes in ulcer therapy, there are incidences of adverse effects and drug interactions during ulcer therapy. Thus, the search for agents having powerful antiulcer activity with natural biological occurrence and predictability, and without side effects is worthwhile[3].

Ziziphus abyssinica A. Rich (*Z. abyssinica*) which has been used by herbalists in northern Nigeria for the treatment of ulcer[4]. The antibacterial, antioxidant and phytochemical screening of the fruit extracts of this plant have been reported[5]. The leaves are applied as poultices and are helpful in liver troubles, asthma and fever[6]. This study was designed to determine the gastro-protective effect of different extracts of *Z. abyssinica* roots, with the aim of establishing the most effective gastro-protective extract.

2. Materials and methods

2.1. Chemicals and reagents

All the chemicals and reagents used were of analytical grade.

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The journal implements double-blind peer review practiced by specially invited international editorial board members.

2.2. Experimental animals

A total of 70 adult albino rats of both sexes weighing between 150 and 200 g were used for the study. They were fed normal rat chow and allowed access to clean water. They were allowed to acclimatize for a period of two weeks before the commencement of experiment. The experimental procedurse used conformed to the United States National Institutes of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research[7] and approved by the Scientific Ethical Committee of ABU, Zaria, Kaduna State, Nigeria.

2.3. Plant material

The roots of *Z. abyssinica* were collected from Faskari, Katsina State, Nigeria. The plant was identified and authenticated at herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria with a voucher number 257.

2.4. Preparation of extracts

The roots were washed, sliced and shade dried before grinding. The powdered sample (300 g) was extracted with hexane, ethyl acetate and methanol by soxhlet apparatus to obtain hexane, ethyl acetate and methanol extracts, respectively. Another 200 g of the powdered roots was soaked in 500 mL of distilled water for 48 h at room temperature and then sieved. The resulting extract was filtered with Whatman No 1 filter paper to obtain the aqueous extract. The extracts were concentrated at 40–45 °C and then stored at 4 °C until used.

2.5. Acute toxicity test

The LD_{50} was carried out according to the organization for Economic and Cooperative Development[8]. Five groups of one rat each was administered graded doses of 3 000 mg/kg body weight. Observation for toxic symptoms was made and recorded at 1, 2, 4, 6 h. The number of survivors noted after 48 h and the toxicological effects were assessed on the basis of mortality.

2.6. Animal grouping

The rats were divided into seven groups. Groups 1, 2 and 3 composed of ten. The rats in groups 4, 5, 6 and 7 were divided into two subgroups of five rats each: one subgroup given the extract at 300 mg/kg, and the other at 600 mg/kg.

Group 1 (control) was given normal rat chow and water with no treatment. Group 2 (control) was received ulcer-induced without pretreatment. Group 3 (standard control) was ulcer-induced and pre-treated with 100 mg/kg cimetidine. Group 4 was ulcer-induced and pre-treated with 300 and 600 mg/kg aqueous extract of *Z. abyssinica*. Rats in group 5 were ulcer-induced and pre-treated with 300 and 600 mg/kg methanol extract of *Z. abyssinica*. Group 6 was ulcer-induced and pre-treated with 300 and 600 mg/kg hexane extract of *Z. abyssinica*. Group 7 was ulcer-induced and pre-treated with 300 and 600 mg/kg ethyl acetate extract of *Z. abyssinica*.

2.7. Induction of gastric ulcer

Rats were pretreated with the extracts for fourteen days and were fasted for 24 h after the last day prior to the administration of ethanol. They were then sacrificed under chloroform anesthesia 1 h after ethanol administration. The stomach of each rat was removed and opened along the greater curvature. Gastric mucosal lesions were measured and scored. The number and severity of erosions were scored using the scoring method as follows: 0: no lesion; 0.5: hemorrhage; 1: 1–3 small lesions < 10 mm length; 2: 1-3 large lesions > 10 mm length; 3: 1–3 thickened lesions; 4: more than 3 small lesions; 5: more than 3 large lesions; 6: more than 3 thickened lesions[9]. The percentage protection was calculated using the following formula:

Protection (%) = (UI Control–UI Treated group × 100)/UI Control

where UI control is ulcer induced control and UI treated group is ulcer induced and treated group.

2.8. Histopathology

Pieces of stomach from each group were fixed in 10% formalin, embedded into paraffin wax, stained with hematoxylin-eosin and examined under microscopic at magnification of 100.

2.9. Statistical analysis

Data were expressed as mean ± SD. The results were analyzed with SPSS version 20 software and One-way ANOVA was employed for comparing the means between groups. The level of significance was set at $P < 0.05$.

3. Results

3.1. Acute toxicity test

The LD_{50} of all extracts of *Z. abyssinica* tested was found to be greater than 5 000 mg/kg. However, a reduced reaction to noise, slow movement and resting at the corner of cages was observed.

The effect of *Z. abyssinica* root extracts on gastric damage induced by absolute ethanol was shown in Table 1. From the result, the ulcer index of rats in the ulcer control group was significantly ($P < 0.05$) higher (4.20 ± 0.68) than all the treated groups, while the control rats, had no trace of ulcer. Pre-treatment of rats with 100 mg/kg cimetidine significantly ($P < 0.05$) decreased the ulcer index to 0.20 ± 0.24 than all other extracts, except the aqueous extract (0.30 ± 0.24) and (0.20 ± 0.24) at 300 and 600 mg/kg, respectively. There was no significant ($P < 0.05$) difference in ulcer index of rats pretreated with cimetidine and those given the aqueous extract. The ulcer index of rats pretreated with methanol (1.40 ± 0.78) and (1.60 ± 0.48) at 300 and 600 mg/kg was significantly ($P < 0.05$) lower than those given the ethyl acetate (2.10 ± 0.58) and (2.30 ± 0.68) at 300 and 600 mg/kg and hexane extract (3.20 ± 1.16) and (3.00 ± 0.89) at 300 and 600 mg/kg, respectively).

Table 1
Effect of Z. abyssinica root extracts on ethanol-induced gastric ulcer.

Groups	Doses (mg/kg)	Ulcer index	Percentage protection
Control	0	0	
Ulcer control	0	4.20 ± 0.68^{e}	-
Standard (cimetidine)	100	0.20 ± 0.24^{d}	95.20
Aqueous extract	300	0.30 ± 0.24^{d}	93.00
	600	0.20 ± 0.24^{d}	95.00
Methanol extract	300	1.40 ± 0.78^{c}	67.00
	600	1.60 ± 0.48^{c}	62.00
Ethyl acetate extract	300	2.10 ± 0.58^{b}	50.00
	600	2.30 ± 0.68^{b}	45.00
Hexane extract	300	3.20 ± 1.16^{a}	24.00
	600	3.00 ± 0.89^{a}	28.00

Results were expressed as mean ± SD. Different subsscripts in the same column are significantly different from control ($P < 0.05$) at 95% confidence level.

*3.2. Effect Z. abyssinica root extracts on acute gastric mucosal lesions induced by ethanol*

Figures 1 (a-f) shows the histological examinations of ulcer control rats and those pre-treated with cimetidine, aqueous, methanol, ethyl acetate and hexane extracts respectively. From the results, rats that were given ethanol without pretreatment (ulcer control group) showed extensive lesions in the gastric portion of stomach (Figure 1a). There were changes to the mucosal epithelium, as well as mucosal necrosis. The epithelium of rats given 100 mg/kg body weight cimetidine was normal (Figure 1b) just as observed in control rats (not presented).

There was slight epithelial change in mucosal lining of rats given the aqueous extract (Figure 1c), while ulceration with changes in mucosal epithelium was observed in rats given methanol extract (Figure 1d). There were no gastric lesions in rats that were pretreated with the standard drug (cimetidine) and the aqueous extract, but there were hemorrhages in some rats. There were intense necrotic changes to apical epithelium in rats given ethyl acetate (Figure 1e) and hexane (Figure 1f) extracts.

4. Discussion

In the present study, the acute toxicity test did not suggest any toxicity or mortality in the Z. abyssinica root extract-treated rats. This shows that the plant is safe and has no toxicity when administered orally up to 5 g/kg. The absence of acute toxicity in rats ensured a good margin of safety for the doses of this extract in the experimental protocols of gastric lesions, as no mortality recorded for each animal[10-12].

Ethanol is considered one of the agents that induces intense gastric ulcers due to altering protective factors[13], such as depletion of gastric mucus and breaking the mucosal barrier, back diffusion of acid, increased gastric mucosal permeability, leading to increased leakage of hydrogen ions from the lumen, and decreased transluminal electrical potential difference[14]. It causes the release of vasoactive mediators such as leukotrienes C4 and histamine making the
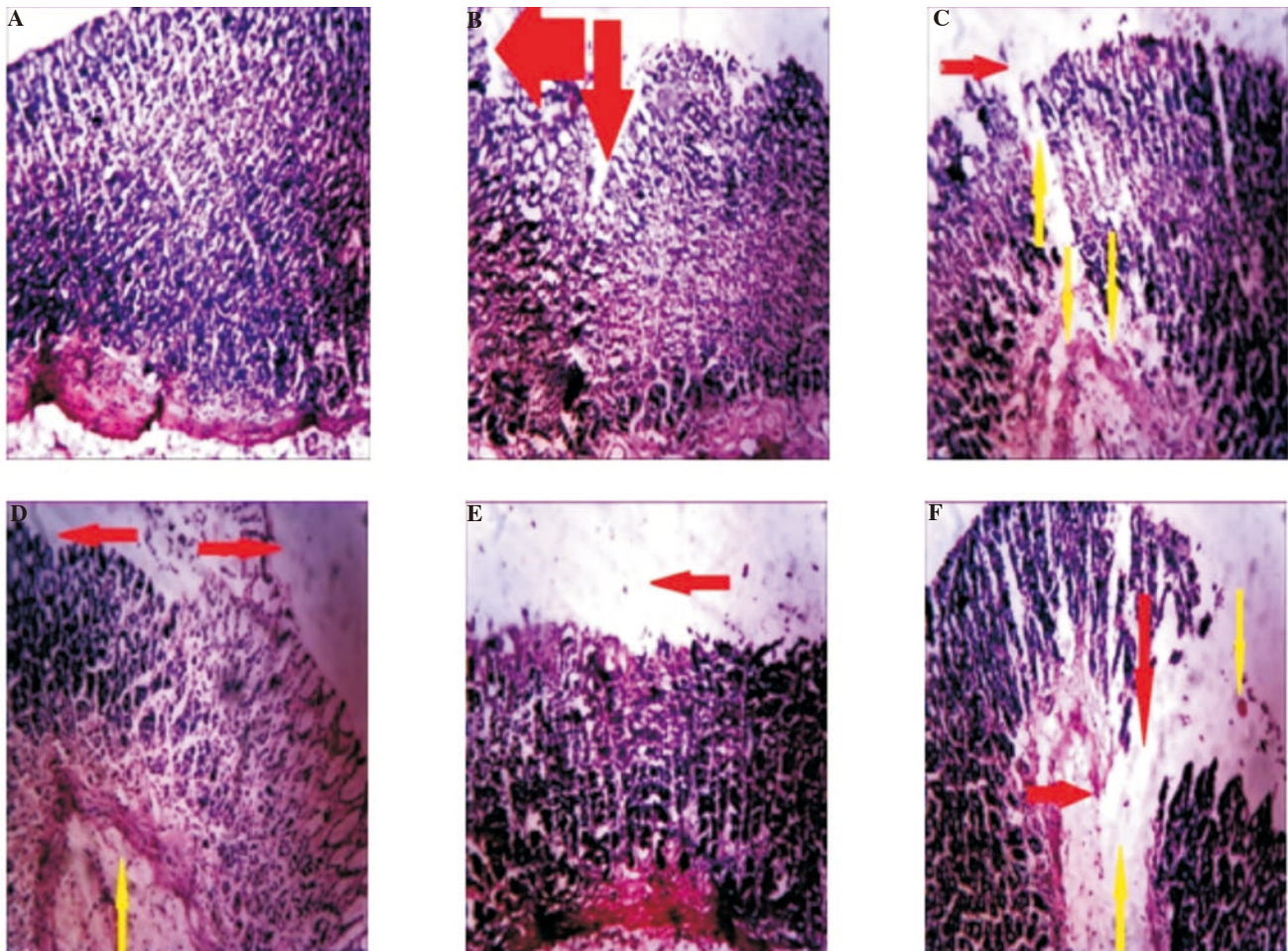
Figure 1. Histological examination of control and experimental animal pretreated with Z. abyssinica root extracts 300 mg/kg body weight.
a: Standard control group; b: Aqueous extract pretreated group; c: Methanol extract pretreated group; d: Ethyl acetate extract pretreated group; e: Hexane extract pretreated group; f: Ulcer control group. The red arrow in the figure shows necrosis; the yellow arrow shows ulceration of gastric tissue (magnification × 100).

submucous membranes constrict with a subsequent blood flow stasis of the mucosa[15,16]. The results suggest that pretreatment with root extract of *Z. abyssinica* markedly ameliorated the ulcer index in ethanol-induced ulcer rats. Administration of the extract for two weeks before ulcer induction has increased the prostaglandin content which is responsible for mucus secretion. The anti-ulcerogenic activity of root extracts of *Z. abyssinica* was demonstrated by Ugwa et al.[17]. The findings also confirmed the cyto-protective nature of the extract as no lesions were seen in rats administered aqueous extract. Such a lower response at higher dose could be due to 'therapeutic windows' effect of the extract[18]. The gastro-protective effect of *Z. abyssinica* root could be due to the presence of biological compounds such as flavonoids, alkaloids, tannins, saponins and other polyphenols present in extracts[19,20]. Alkaloids have been shown to possess gastro-protective and ulcer healing activity by inhibiting acid secretion and gastric motility, stimulation of mucus/bicarbonate secretion and increase mucosal blood flow[21]. Tannins precipitate microproteins at the site of injury forming protective layer that prevents absorption of toxic substance and promote mucosa resistance to the action of proteolytic enzymes. Through these, tissue repair is enhanced and oxidative damage prevented[22]. Flavonoids have been shown to increase the mucosal content of prostaglandins and mucus in gastric mucosa showing cytoprotective effect against necrotic agents. These plant constituents present in *Z. abyssinica* root extracts might have the ability to protect against ulceration induced by ethanol.

In conclusion the present study shows that aqueous extract of *Z. abyssinica* root was more effective against experimentally induced gastric ulcer. This justifies its use in the treatment of gastrointestinal diseases in the northern part of Nigeria.

Conflict of interest statement

The authors report no conflict of interest.

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