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EVALUATION OF THE ANTI-ULCER ACTIVITY OF AQUEOUS STEM-BARK EXTRACT OF *HYMENOCARDIA ACIDA* (FAMILY – EUPHORBIACEAE)

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Abstract
To assess and establish the scientific basis for the anti-ulcer activity of aqueous stem-bark extract of *Hymenocardia acida*. Groups of albino rats were pre-treated orally with aqueous stem-bark extract of the plant before administration of indomethacin and induction of cold-restraint stress ulcers by immobilizing each animal in a cylindrical cage maintained at 2 to 4°C in a refrigerator for 3 hours. The animals were sacrificed by a blow on the head, their stomachs removed and examined for ulcers. The extract produced significant ($P< 0.05$) anti-ulcer activity against the two models studied. The anti-ulcer activity against cold-restraint stress was also dose-dependent. Phytochemical studies revealed the presence of glycosides, saponins and tannins. This study demonstrates that the plant extract has significant anti-ulcer activity against experimentally induced gastric lesions.

Keywords: Antiulcer activity*: Hymenocardia acida;* Rats

INTRODUCTION

Hymenocardia acida (Family-Euphorbiaceae) is a common savanna plant usually a shrub, with twisted branches and orange-brown bark. It is widespread in tropical Africa. The morphological characteristics have been described in literature[1,2]. Some reported traditional uses include: colic pains, ulcers, wounds, trachoma, fever, as aphrodisiac and as analgesic.[3] However, to the author's knowledge, there are no pharmacological studies reported in literature about the stem-bark of this plant which herbalists in different parts of Enugu State of Nigeria use for the treatment of various acute stomach disorders. A herbalist Chief Edwin Nweze from Okpuje in Enugu State who supplied this "drug" claimed that the aqueous extract of the bark was very effective in the treatment of all cases of bleeding ulcers, colic pain, diarrhea, dysentery and low abdominal pain due to menstruation in the female. The present study was therefore carried out to: identify pytochemically the main active constituents of the aqueous extract of the stem-bark, and assess and/or confirm pharmacologically, the apparent effects on bleeding ulcers.

MATERIALS AND METHODS

Identification

The plant was identified by the staff of the Department of Pharmacognosy, University of Nigeria, Nsukka. A voucher specimen has been preserved in the herbarium for future reference.

Preparation of the extract

The stem-bark was collected in October, 2002 by Mr. A. Ozioko of the Botany Department of the same University. The bark was cleared

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of all debris, cut into smaller pieces, dried under shade and pulverized. Distilled water was used to macerate the pulverized bark overnight. The extract was prepared in such a way that 1 ml of extract was equivalent to 0.05g of the dried bark.[4] The extract was stored in a refrigerator for future use throughout the study.

Animals

Inbred albino mice of both sexes (20-25g) were used for acute toxicity test (LD_{50}), while inbred male albino rats (180-230g) were used for other animal experiments. Food was withdrawn 24 hours before each experiment and replaced with a solution of 8% dextrose in 0.2% sodium chloride to maintain adequate hydration and metabolism.[5] The solution was withdrawn one hour before drug treatment. Animals to be stressed were put into individual close-fitting tubular restraint cages of wire mesh and exposed to a temperature of 2-4^{o} for 3 hours in a refrigerator.

Phytochemical Screening

The aqueous extract was tested for the presence or absence of alkaloids, glycosides, saponins, flavonoids, tannins and fats ad oils using standard phytochemical procedures and tests.[6]

Acute toxicity test (LD_{50})

Food was withdrawn from the animals (mice) 24 hours before the experiment and one hour to treatment dextrose in saline solution was withdrawn. Trial experiments were undertaken by administering widely differing doses (10, 100, 500, 1000 mg/kg) of the extract dissolved in normal saline to give a constant volume of 5 ml/kg orally to 4 groups of 3 animals each divided at random. The lowest dose that killed the groups of mice was used as the reference point for predicting the dose range that might be used.[7] For the main experiment 5 groups of 6 mice each were administered orally the predetermined doses of the extract (200, 300, 400, 500 and 600 mg/kg). The mice were observed for 24 hours and the LD_{50} was calculated.[8]

Anti-ulcer activity.

Two models (indomethacin and cold-restraint stress) for inducing acute experimental ulcer lesions in laboratory animals were used to evaluate the anti-ulcer activity of the extract. Thirty rats were used for indomethacin model and forty for cold-restraint stress model and they were divided into three or four groups of 10 each respectively. Group A and group C had 10 animals each and served as negative and positive control as they received saline (5 ml/kg) and cimetidine (100 mg/kg respectively.

Indomethacin-induced ulcers

Food was withdrawn from 30 rats, and one hour prior to treatment dextrose in saline solution was withdrawn. They were randomly divided into three groups and coded to prevent observer bias. Saline (5 ml/kg), the extract and cimetidine were administered in the doses and manner described above. One hour later, indomethacin 30 mg/kg was administered orally to all the rats.[9] After seven hours of indomethacin administration, the rats were killed by a blow on the head. The stomachs were removed and each opened along the greater curvature. After fixing the tissue by immersing in 10% formalin, it was rinsed under a stream of water and examined for ulcers. The ulcers were counted by the aid of a hand lens (x 10 magnification), and given severity rating as follows: less than 1 mm = 1, 1 – 2 mm = 2 and greater than 2 mm = 3. The overall total was divided by a factor of 10 to derive the ulcer index for each animal.

Stress-induced ulcers.

Forty fasted rats were randomly divided into four groups of 10 each. The four groups were then pre-treated orally with saline (5 ml/kg), the extract at doses of 50,g and 100 mg/kg and cimetidine respectively. One hour after drug administration, cold restraint stress was induced in the animals.[10] The animals were immobilized and placed in a refrigerator at 2-4^{o}C for 3 hours. At the end of the period, the animals were removed from the cold,

sacrificed 30 minutes later. The stomachs were opened and studied as described before.

Statistical analysis

The results are shown as the mean ulcer index ± standard error of the mean. The significance of the data was evaluated using Student's t-test.

RESULTS AND DISCUSSION

Phytochemical Screening

The results of the phytochemical screening indicate that the extract contains saponins, glycosides and tannins. Alkaloids and flavonoids were absent.

Acute toxicity

The LD_{50} of the extract was found to be 483.84 mg/kg

Anti-ulcer activity.

Indomethacin induced ulcers. (Table 1). As shown in the Table, indomethacin induced ulcers in 100% of the animals in the negative control group. The ulcer index was 4.60 ± 1.09. Pre-treatment with the extract and cimetidine significantly ($p<0.05$) reduced the ulcerogenic potentials of indomethacin. Only 40% of the group, which was pre-treated with the extract, and 60% of those pre-treated with cimetidine developed gastric ulcers induced by indomethacin. The ulcer index was reduced to 0.28 ± 0.22 and 1.02 ± 0.74 respectively. The anti-ulcer activity of the extract was even greater than that of cimetidine and the difference was significant ($p<0.05$).

Stress-induced ulcers (Table 2). Ninety percent of the animals in the negative control group developed ulcers. At a dose of 50 mg/kg, the extract exhibited 75.93% protection and the ulcer index was reduced from 0.54 ± 0.28 to 0.13 ± 0.06. At a dose of 100 mg/kg body weight, all the ten animals used were protected as none developed any lesions. Although only 4 out of the 10 animals pre-treated with cimetidine developed ulcers, the percentage protection was only 9.26 and the difference in ulcer index was not significant ($p>0.05$).

Table 1: Effects of the aqueous extract of *Hymemocardia acida* on Indomethacin-induced ulcers in rats

Group	Treatment	Quantal ulcer incidence	Mean ulcer index+SEM	Percentage protection (%)[1]
A	Saline (5 ml/kg)	$^{10}/_{10}$	4.60 ± 1.09	0.00
B	Extract (50 mg/kg)	$^{4}/_{10}$	0.28 ± 0.22	93.91*
C	Cimetidine (100 mg/kg)	$^{6}/_{10}$	1.02 ± 0.74	77.83*

* = Significant

[1] Percentage protection to ulcer formation in rats by the extract was calculated as follows:

$$\left\{1-\left[\frac{Mean\ Ulcer\ index\ with\ extract}{Mean\ Ulcer\ index\ with\ saline\ (5ml/kg)}\right]\right\}\times 100$$

Table 2: Effects of the aqueous extract of *Hymenocardia acida* on cold-restraint (stress) - induced ulcers in rats.

Group	Treatment	Quantal ulcer incidence	Mean ulcer index±SEM	Percentage protection (%)[1]
A	Saline (5 ml/kg)	$^{9}/_{10}$	0.54 ± 0.28	0.00
B_1	Extract (50 mg/kg)	$^{3}/_{10}$	0.13 ± 0.06	75.93*
B_2	Extract (100 mg/kg)	$^{0}/_{10}$	-	100.00*
C	Cimetidine (100 mg/kg)	$^{4}/_{10}$	0.49 ± 0.08	9.26

* = Significant

[1] Percentage protection to ulcer formation in rats by the extract was calculated as follows:

$$\left\{1-\left[\frac{Mean\ Ulcer\ index\ with\ extract}{Mean\ Ulcer\ index\ with\ saline\ (5ml/kg)}\right]\right\}\times 100$$

The aqueous extract of *Hymenocardia acida* exhibited significant anti-ulcer effects. It significantly inhibited indomethacin and cold restraint stress-induced gastric ulcers in rats. The protective effect of the aqueous extract against stress-induced ulcer was dose-dependent and was more pronounced than with indomethacin. Its protective effects also compare favourably to cimetidine, an effective H_2-receptor antagonist. The LD_{50} of the extract was 483.84 mg/kg while the minimum effective dose was 50 mg/kg. This showed that it has a wide safety margin.

The mechanism of action by which the extract prevents development of ulcer in rats has not been elucidated. However, pharmacological screening on isolated tissue preparations is in progress to elucidate the possible mechanisms of action and preliminary observations in this area are encouraging. The pathogenesis of ulcer disease is believed to involve the interplay of two distinct components – aggressive factors (such as acid, pepsin) and defensive factors (such as mucosal barrier and mucus).[11] Phytochemical screening revealed the presence of saponins and tannins. Some saponins like glycyrrhizic acid of liquorice and their triterpene derivatives like carbenoxolone[12] have been found to be effective in ulcer healing by forming protective mucus materials on the gastric mucosa.[13] Since the extract contains mainly saponins and tannins, they might have been responsible for the observed effects. The astringent property of the tannins present might contribute to the antiulcer activity.

CONCLUSION

The results of this study show that the aqueous extract of *Hymenocardia acida* is effective against experimentally induced gastric ulcers in rats as studied with indomethacin, and cold restraint stress. The extract appears to be potentially useful in the treatment of gastric disorders (bleeding ulcers). This may confirm the medicinal benefits derived from and claim of the herbalists. The extract has a wide margin of safety in mice. The major constituents appeared to be saponins and tannins. The mechanism of action by which this extract offers protective effects is being explored by further work in progress.

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