Journal of Applied Pharmaceutical Science Vol. 3 (02), pp. 139-142, February, 2013
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2013.30224
ISSN 2231-3354

Anti-ulcer activity of *Bombax buonopozense* P. Beauv. aqueous leaf extract (Fam: Bombacaceae)

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ARTICLE INFO

Article history:
Received on: 11/01/2013
Revised on: 29/01/2013
Accepted on: 19/02/2013
Available online: 28/02/2013

Key words:
Anti-ulcer activity, *B. buonopozense*, leaves, phytochemical analysis.

ABSTRACT

The objective of this study was to investigate the anti-ulcer activity of leaves of *Bombax buonopozense* P. Beauv. (Bombacaceae) in rats. Fresh dried leaves of *B.buonopozense* were extracted by cold maceration which yielded a mucilaginuous aqueous extract. Anti-ulcer effects of the aqueous extract at 100, 200 and 400 mg/kg were evaluated in rats using ethanol-induced ulcer model. Phytochemical analysis and lethality tests (LD_{50}) were carried out using standard procedures. Results showed that the aqueous extract exhibited significant ($P<0.05$) and dose- dependent anti-ulcer activity in the model used. Percentage ulcer inhibitions of extract at 100, 200 and 400 mg/kg for ethanol-induced ulcers were 39.76, 62.07 and 75.73% respectively. Ulcer protection in the model used by the extract is dose-dependent and the ulcer inhibitory effects of the extract are comparable to ranitidine. Oral LD_{50} value is 2828.42 mg/kg in mice. Phytochemical analysis showed the presence of flavonoids, saponins, tannins, resins, balsams, carbohydrates, oligosaccharides, terpenes, reducing sugars and sterols. Therefore, results of our study suggest that the aqueous extract of *B. buonopozense* possesses anti-ulcer activity as claimed by its folkloric use.

INTRODUCTION

The exact pathogenesis of ulcer continues to elude scientists and medical researchers, but a common ground has been proposed. Ulcers are produced when any factor causes an imbalance between the protective factors (mucus and bicarbonate) and aggressive factors (acid and pepsin) in the stomach (Ojewole, 2004; Del Valle et al., 2003). Such factors could range from natural causes (gastric cancer), infections (*H.pylori*), lifestyle (drugs- non-steroidal anti-inflammatory agents, alcohols, stress and cigarette smoking) (Berardi and Welage, 2005; Suerbaum and Michetti, 2002). Current treatment of ulcers in developing countries has been largely suppressing pain, with little or no strategy aimed at a cure. Herbal medicine is fast emerging as an alternative treatment to available synthetic drugs for treatment of ulcer possibly due to lower costs, availability, fewer adverse effects and perceived effectiveness. Many tropical herbs have been scientifically reported to possess potent anti-ulcer activity as proposed by earlier workers (Vela et al., 1997; Goulart et al., 2005; Singh et al., 2008; Aguwa and Ukwe, 1997). *B. buonopozense* is a large tropical tree that grow to 40 metres in height with large buttress roots that can spread 6 metres. The individual leaflets have entire margin and white large, measuring from 8 to 23 cm in length by 3 to 7. 5 cm in width with the undersides of the leaflets being either glabrous or puberulous and conical buds which contains many seeds that are 5 to 6 mm in length, all of which have a cotton-like fibre covering (Beentje and Sara, 2001). The plant is widely distributed in African countries such as Ghana, Sierra Leone, Uganda, Gabon and different parts are used for different purposes (Dubost, 1984). *B. buonopozense* (Akpu in Igbo, Gurjiya in Hausa and Ogbolo in Yoruba) is a wild plant whose edible floral part is used as vegetable by the inhabitants of North Central, Nigeria and as medicine due to its nutritive and therapeutic properties (Mann et al., 2003). The plant has been reported in literature to possess anti-microbial activity by its edible floral extracts (Mann et al., 2011). Earlier studies also have reported the anti-diarrheal activity of its methanolic leaf extracts (Akuodor et al., 2011). In Nupe/Gbagyi of Nigeria, leaves of this plant is claimed to be effective in the treatment of diarrhea, dysentery, "hot

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stomach" and leprosy (in combination with other herbs) especially when taken as an aqueous decoction (Irvine, 1961). This study was designed to evaluate the anti-ulcer activity of the plant and to identify phytochemically the constituents of the extract responsible for the observed activity.

MATERIALS AND METHODS

Collection and identification of plant material

The leaves of *B. buonopozense* were collected in large quantities from Dei-Dei forests, Abuja in April 2009 and was identified by Mrs. N.A. Ugbabe of the Herbarium Unit, NIPRD Abuja. A voucher specimen has been deposited at the herbarium of the National Institute for Pharmaceutical Research and Development (NIPRD), Idu Abuja with voucher number NIPRD/H/6405 and in the herbarium of the Department of Pharmacognosy and Environment Medicine, University of Nigeria, Nsukka with voucher number UNN/PCG/09/045 for future reference.

Preparation of extracts

300 g of shade-dried coarse powered leaves was macerated overnight in distilled water. The mixture was filtered and the mucilaginous greenish extract was stored in refrigerator for subsequent use. The extract is allowed to assume room temperature prior to use for analysis.

Phytochemical screening

The aqueous extract was tested for the presence or absence of secondary metabolites using standard phytochemical procedures and tests (Harbourne, 1984).

Animals

In-bred albino mice (21-27 g) and rats (100-170 g) of both sex supplied by the staff of the Department of Zoology, University of Nigeria, Nsukka were used. They were housed in steel cages, placed on standard pellet feed (Niger-feed, Nigeria) and were given free access to clean water. They were kept in well ventilated rooms with a 12/12 h light/dark conditions and ambient room temperature. Animals were obtained two weeks before the experiments to acclimatize with the laboratory environment. Animal experiments were done in compliance with the National Institute of Health Guide for care and use of laboratory Animal (Pub.No.85-23, revised 1985).

Acute toxicity and Lethality tests

The acute toxicity profile of the extract was assessed using standard procedures (Lorke, 1983). Three groups of 3 mice each were administered 10, 100 and 1000 mg/kg of the aqueous extract orally. The mice were observed for 24 h for effects of toxicity and the number dying in each group within the period was noted. When no deaths were recorded, another three groups of 3 mice each were administered 1600, 2900 and 5000 mg/kg of the extract orally. The animals were observed for 48 h for effects of toxicity and the number dying in each group within the period was recorded.

Anti-ulcer activity

The model, ethanol-induced ulcer in rats was employed to evaluate the anti-ulcer activity of the aqueous leaf extract of *B. buonopozense* using standard method (Dordevic et al., 2007). All the rats used were fasted for eighteen hours but were given water freely till the start of the experiment.

Ethanol-induced ulcer

Thirty fasted animals were used in five groups of six animals each. Groups A and B received 2 ml/kg distilled water (negative control) and 20 mg/kg p. o. ranitidine (Peptard®, Neimeth) respectively while rats in groups C, D and E were given 100, 200 and 400 mg/kg of the extract orally (p.o) respectively. After one hour, all animals received 0.5 ml/kg of 70 % ethanol (Sigma-Aldrich, Germany) orally. The rats were sacrificed with chloroform (Sigma-Aldrich, Germany) anaesthesia after one hour. The stomachs were isolated, washed gently under clean flowing water and cut open along the greater curvature. The stomachs were then fixed in 10 % formalin and craters observed and ulcer scores were recorded using standard method (Vogel et al., 2002).

Ulcer Index (UI): UI was calculated using the formula:

$$UI = U_N + U_S + U_p \times 10^{-1}$$

Where, U_N=No. of ulcers/animals, U_S= Mean severity of ulcer scores and U_p=Percentage of animals with ulcer incidence.

Percentage protection of ulcer

Percentage Protection was calculated using the formula:

$$\text{Percentage Protection} = \frac{Control\ (UI) - Test\ (UI)}{Control\ (UI)} X\ 100$$

Statistical analysis

Ulcer indices were shown as the mean ± standard error of mean (SEM) and level of ulcer protection presented as percentage inhibition. The significance of the differences in mean ulcer indices between extract and negative control was calculates at 95 % confidence interval using student's t-test.

RESULT

Phytochemical screening revealed the presence of saponin, tannins, flavonoids, resins, terpenes, carbohydrates, oligosaccharides, balsams, reducing sugars and sterols. Acute toxicity results showed that the LD_{50} of the aqueous is 2828.42 mg/kg, therefore the extract is safe up to a maximum dose of 2800 mg/kg.

Ethanol-induced ulcer

In the table below, ulcer inhibition was evident in all treatment of the aqueous extract of *B. buonopozense* compared to the negative control. However, statistically significant ulcer inhibition (62.07 and 75.73 %, $p < 0.05$) could be seen only at doses of 200 mg/kg and 400 mg/kg of the aqueous extract. The protection from ulcer was dose dependent even as ulcer was produced in all rats in this model.

Table. 1 Effects of Aqueous Leaf Extract of *B. buonopozense* on Ethanol-induced Ulcers in Rats (n=6).

Treatments	Dose mg/kg p. o	Quantal ulcer incidence	Ulcer index	Ulcer inhibition (%)
Distilled water	2 ml/kg	6/6	1.90± 0.15	0
Ranitidine	20	1/6	0.52± 0.09	70.73
Extract	100	6/6	1.40± 0.22	39.76
Extract	200	6/6	0.65± 0.09*	62.07
Extract	400	1/6	0.50± 0.09*	75.73

Ulcer indices are expressed as Mean ± SEM: n= number of animals in each group. *$p< 0.05$ v_s negative control (student t-test).

DISCUSSION

The anti-ulcer activity of the aqueous leaf extract of *B. buonopozense* against ethanol-induced ulcer was established in this study. Results of acute toxicity showed that the plant is safe up to a maximum dose of 2800 mg/kg. The extract protected the stomach against ethanol's necrotic damage and its effect at 400 mg/kg was comparable to ranitidine- an anti-secretary agent (due to its H_2 –receptor antagonistic effect) at 20 mg/kg. Ethanol challenge induces gastric injury due to production of oxygen free radicals leading to increased lipid peroxidation, which causes damage to cell and cell membrane presenting as red streaks of sores (Pihan et al., 1987).

The protection by the extract of this type may suggest a possible anti-secretary mechanism of action due to H_2-receptor antagonism. This results to a sustained reduction in mucosal blood flow and a subsequent generation of ulcer. Ranitidine was employed in this study for its anti-secretary effect as it indirectly suppress gastric acid and pepsin secretion by occupying H_2–receptors; hence ranitidine exhibits an anti-secretary and protective effect against ulcers (Aguwa, 2004).

The presence of saponins, tannins, flavonoids, terpenes, and steroids in this extract as seen in this study has also been reported by earlier studies (Akuordo et al., 2011; Iwu, 1993). Ulcer protection may be attributed to any of these phytochemical constituent as flavonoids, tannins and saponins which have been shown to produce anti-ulcerogenic and anti-gastric activity (Carlo et al., 1994; Aguwa and Ukwe, 1997). However, until specific constituents are isolated and characterized, exact mechanism of action cannot be ascertained.

CONCLUSION

We have demonstrated in this study that the aqueous leaf extracts of *B. buonopozense* has an ulcer healing property against experimentally induced ulcers in rats and this study confirms folkloric claims of the benefits of *B.buonopozense* in treatment of ulcer. The results also suggest that the anti-ulcer activity is probably due to possible involvement of mucus in anti-ulcer-effect of extracts, or probably by its free radical scavenging effect or may be also due to its anti-secretary activity.

ACKNOWLEDGMENT

Special appreciation goes to the staff of the National Institute for Pharmaceutical Research and Development (NIPRD), Idu Abuja and post graduate laboratory Department of Pharmacognosy and Environmental Medicine, University of Nigeria Nsukka.

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How to cite this article:

Nwagba, C. A; Ezugwu, C.O; Eze, C.C; Anowi, F.C; Ezea, S.C; Nwakile, C. D. Anti-ulcer Activity of *Bombax Buonopozense* P. Beauv. aqueous leaf Extract (Fam: Bombacaceae). J App Pharm Sci. 2013; 3 (02): 139-142