

Full Length Research Paper

Evaluation of anti-ulcer and antimicrobial effects of *Verbena hastata* leaf extract

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Accepted 14 April, 2011

The ulcer protective and antimicrobial effects of the ethanolic leaf extract of *Verbena hastata*, a popular herbal traditional medicine in southern Nigeria, were assessed using ethanol and indomethacine induced gastric ulcer in rats and against some disease causing microorganisms, respectively. The extract (100-400 mg/kg, *p.o*) exerted ulcer-protective activities against ethanol and indomethacine-induced ulceration in rats with maximum anti-ulcer effect observed at 400 mg/kg. In addition, *V. hastata* leaf extract (2.00 mg/ml) showed absence of antimicrobial effects against all the tested organisms. The oral LD₅₀ values obtained were greater than 5000 mg/kg in mice. The results clearly indicate that *V. hastata* leaf extract possesses potent ulcer protective properties. It might be a useful contribution to highlight the mechanism of action of this plant as anti-ulcer agent.

Key words: Antimicrobial, *Verbena hastata*, antiulcer agent, traditional medicine.

INTRODUCTION

Medicinal plants are believed to be important sources of new chemical substances with potential therapeutic effects. Natural medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, though relatively little knowledge about their mode of action is available. There have been reports that a vast majority of the population particularly those living in villages depend largely on herbal medicines (Gupta, 1994). Before the availability of synthetic drugs man was completely dependent on natural medicinal plants for curing diseases (Singh et al., 2008). These plants, which abound in our environment, enjoy wide

acceptability by the population and serve as cheaper alternatives to orthodox medicine (Sofowora, 1993; Akah and Nwabie, 1994). There is a growing interest in the pharmacological screening of various plants used in Nigerian traditional systems of medicine.

Verbena hastata L. (Family: Verbenaceae) is a bristly perennial commonly found in Southern Nigeria. The quadrangular stem reaches a height of two to five feet and bears leaves that are oblong and lanceolate serrate and three to six inches long. Different parts of the plant, especially the stem, root, and leaves are widely employed in traditional medicine. Extract of the leaves in water or diluted alcohol has been used in the treatment of dysentery, diarrhoea (Akuodor et al., 2010e) and pains (Akuodor et al., 2011). It has been used as a remedy for malaria treatment (Akuodor et al., 2010c), colds and lung

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congestions. The herb has also been used as an antiperiodic, diaphoretic, expectorant and is often effective in the elimination of intestinal worms.

Verbena species have been used as medicinal plants in folk medicine against ascaris, demulcent, for constipation, for menstrual flow, milk secretion, herpes zoster, sedative, snake bite, stomach-ache, tranquilizer (Akerrete et al., 2007; Teklehaymenot and Giday, 2007; Al-Qura'n, 2009; Yapici et al., 2009; Cakilcioglu and Turkoglu, 2010). However, no work has been reported on anti-ulcer and antimicrobial effects of the plant. In view of this, the present study was designed to investigate the ulcer protective and antimicrobial potentials of *V. hastata* leaf extract.

MATERIALS AND METHODS

Plant collection and extraction

Fresh leaves of *V. hastata* were collected by Mr. Joseph L Akpan at Nnug Ita village, Akwa Ibom State, Nigeria. The plant was identified and authenticated by Prof. J.C Okafor of University of Nigeria, Nsukka. The leaves were cleaned and air-dried at room temperature for 7 days and crushed into coarse powder using mortar and pestle. Two hundred and twenty grams of the powdered material was macerated with ethanol for 24 h with constant shaking. The liquid extract obtained was concentrated to dryness in vacuum at 40°C. The yield was (8.2% w/w).

Experimental animals

Adult Wistar rats of both sexes weighing between (180-250 g) were used for the studies. The animals were maintained in the Animal Facility Centre, Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development, Abuja, Nigeria. The animals were kept in cages at room temperature and moisture, under naturally illuminated environment of 12:12h dark/light cycle. They were fed with NIPRD formulated feeds and had access to water *ad libitum*. All animal experiments compiled with the "Principles of Laboratory Animal Care" (NIH Publication No. 85-23, revised in 1985) and NIPRD-Standard operation procedure.

Test organisms

The microorganisms used for the screening include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans* and *Escherichia coli*. The organisms were clinical isolates obtained, standardized and stored by the Department of Microbiology, Human Virology and Biotechnology, National Institute for Pharmaceutical Research and Development, Abuja, Nigeria.

Chemicals

Ranitidine (Ranbaxy, UK.). Indomethacine (Sigma, USA), Ethanol (Sigma-Aldrich GmbH, Germany) Nutrient agar (BBL, USA) and Nutrient broth (BBL, USA) were used for the investigation.

Phytochemical tests

The ethanol leaf extract of *V. hastata* was subjected to standard phytochemical analyses for different constituents (Trease and

Evans, 1983). The extract was tested for the presence of alkaloids, flavonoids, saponins, tannins, terpenes, sterols and carbohydrates.

Acute toxicity study

Acute toxicity study was carried out according to the modified method described by Lorke, 1983. The study was carried out in two phases. In the first phase, nine mice were randomized into three groups of three mice per group and given 10,100 and 1000 mg/kg *p.o*, respectively. Animals were observed for 72 h after treatment for signs of toxicity and mortality. The results of this phase provided data for the choice of dose for second phase, in which 1600, 2900 and 5000 mg/kg were administered orally to another fresh set of three mice per group. The animals were also observed for signs of toxicity and mortality. The final LD₅₀ value was calculated as the square root of the product of the lowest lethal dose and the highest non-lethal dose, that is, the geometric mean of the consecutive doses for which 0 and 100% survival rates were recorded in the second stage.

Ethanol-induced ulceration

The animals were fasted for 24 h but had water *ad libitum*. The rats were grouped into 5 (n= 6). Group 1 received 10 ml/kg normal saline. Group 2 received 20 mg/kg of Ranitidine, standard drug. The remaining three groups received 100, 200 and 400 mg/kg of *V. hastata* leaf extract, *p.o*. One hour later, ulceration was induced by intragastric instillation of 1 ml of 90% ethanol and one hour after ethanol administration, the animals were sacrificed under ether anaesthesia and the stomach were surgically removed and opened along the greater curvature to microscopically examine any ulcerative lesions. The number, length and severity of the ulcers were noted and scored on an arbitrary 0-6 point scale (Magistretti et al., 1988). The scores were as:

- 0 = No lesion
- 1 = 1-3 small lesions
- 2 = 1-3 large lesions
- 3 = 1-3 thick lesions
- 4 = more than 3 small lesions
- 5 = more than 3 large lesions
- 6 = more than 3 thick lesions

Indomethacine -induced gastric ulceration

The animals were fasted for 24 h having access to water *ad libitum*. The rats were grouped into 5 (n=6). Group 1 received 10ml/kg normal saline, standard drug. The remaining three groups received 100, 200 and 400 mg/kg of *V. hastata* leaf extract, *p.o*. One hour later, ulceration was induced by oral administration of 25 mg/kg of indomethacine. Five hours later, animals were sacrificed under ether anaesthesia and the stomach were surgically removed and opened along the greater curvature to microscopically examine any ulcerative lesions. Ulcer lesions were scored according to severity of ulceration as described by Asuzu and Onu, (1990), Akah et al. (1998):

- < 1 mm (Pin point) =1
- 1mm (Pin point) = 2
- >-2 1mm (Pin point) = 3
- >-3 1mm (Pin point) = 4

Antimicrobial studies

Agar dilution method was used to determine the antimicrobial

Table 1. Effect of the leaf extract of *V. hastata* on ethanol-induced gastric ulceration in rats.

Group	Dose (mg/kg)	Ulcer index (UI)	Maximal protection of ulceration (%)
Control	Normal saline	3.6 ± 0.52	-
Ranitidine	20	0.33 ± 0.21	91 *
<i>V. hastata</i>	100	2.33 ± 0.56	37 *
	200	1.00 ± 0.52	73 *
	400	0.5 ± 0.34	86 *

*Significant (P < 0.05) compare to control.

Table 2. Effect of the leaf extract of *V. hastata* on indomethacine-induced gastric ulceration in rats.

Group	Dose (mg/kg)	Ulcer index (UI)	Maximal protection of ulceration(%)
Control	Normal saline	3.5 ± 0.22	-
Ranitidine	20	0.33 ± 0.33	91 *
<i>V. hastata</i>	100	2.67 ± 0.41	24 *
	200	1.5 ± 0.22	57 *
	400	0.83 ± 0.40	76 *

*Significant (P < 0.05) compare to control.

activity (Jones et al., 1989). The inoculums for each organism *S. aureus*, *P. aeruginosa*, *E. coli* and *C. albicans* were prepared from broth cultures containing approximately 5×10^6 to 9×10^7 colony forming units (CFU) in 1 ml.

The diluted (1:20) inoculum was applied as a streak with a loop calibrated to deliver 0.002 ml and containing 9×10^3 CFU. The plates were incubated for 24 h at 37°C. The plant extract was prepared at a concentration of 2 mg/ml. The extract was dissolved in sterile water which was also used as control. Observations were performed in duplicates.

Statistical analysis

The results were expressed as mean ± S.E.M. statistical analysis was carried out using analysis of variance (ANOVA), followed by tuckey's test and difference between means were considered significant where $P \leq 0.05$ (Kirkwood et al., 2003).

RESULTS

Phytochemical test

The phytochemical screening of *V. hastata* leaf extract revealed the presence of Saponins, Tannins, Flavonoids, Steroids, Terpenes and Carbohydrates.

Acute toxicity test

There was no mortality observed upon oral administration in mice, even at doses as high as 5000 mg/kg. Hence, the LD₅₀ in mice can be considered to be greater than

5000 mg/kg. Apart from mild weakness and drowsiness, *V. hastata* did not produce any major clinical signs of toxicity in mice during a 4-day observation period.

Ethanol induced gastric ulcer effect

V. hastata leaf extract was found to possess remarkable (P < 0.05) ulcer-protective effect of 37 and 73% inhibition at 100 and 200 mg/kg, while the maximum effect of 86% inhibition of ulceration was observed at 400 mg/kg. The standard drug (Ranitidine) produced 91% protection as shown in Table 1.

Indomethacine-induced gastric ulcer effect

The ethanol leaf extract of *V. hastata* remarkably (P < 0.05) inhibited ulceration induced by indomethacine dose-dependently. The maximum ulcer protection was observed at 400 mg/kg while the standard drug (Ranitidine) gave 91% ulcer protection (Table 2).

Antimicrobial activity of the extract

The results of the antimicrobial activity of the ethanol leaf extract of *V. hastata* (2.00 mg/ml) revealed that the extract did not inhibit the growth of all the tested organisms. Distilled water used as control was inactive against the test organisms.

DISCUSSION

The present study shows that *V. hastata* possess a significant antiulcer activity as evident from significant decrease in ulcer index at all doses in both the experimentally-induced gastric ulcer models. Ulcer formation induced by indomethacine is known to be related with inhibition of cyclooxygenase that prevents prostaglandin biosynthesis, which in turn inhibits the formation of mucus, a defensive factor against gastrointestinal damage (Bandyopathy et al., 2000). The production of gastric lesions is one of the significant side effects of non-steroidal anti-inflammatory drugs (Pagella et al., 1983). The ulcerogenic action of indomethacine especially in an empty stomach has already been established (Rasool et al., 2008). The ulcerogenic effect of NSAIDs seems to be related to the inhibition of endogenous PGs synthesis; although it has also been established that indomethacine modifies other protective mechanisms of the gastric mucosa, including gastric secretion and the permeability of the gastric mucosal barrier also (Rainford, 1978). Indomethacine is known to induce a relative increase in leukotriene C4 at the cost of reduced PGE₂ levels which may induce mucosal vasoconstriction and enhance NSAIDs-induced injury (Hawkey, 1989). In addition, indomethacin is also known to inhibit the cyclooxygenase enzyme (COX) responsible for the production of prostaglandins, involved in general house keeping activities, e.g. maintenance of gastric mucosal integrity (Rasool and Varalakshmi, 2006). Inhibition of COX-1 enzyme may result in the formation of ulcers in many human and hence the selective inhibition of COX-2 enzyme by compounds has a major advantage over non-selective non-steroidal anti-inflammatory drugs (Smith et al., 2000).

Ethanol and indomethacine restraint stress are among the most commonly utilized experimental models for the evaluation of anti-ulcer activity in rats (Lewis and Hanson, 1991; Akah et al., 1997). Ethanol-provoked gastric mucosal lesions are caused by the direct toxic effect of ethanol through the reduction in mucus production, gastric mucosal blood flow and bicarbonate secretion. Endogenous glutathione and prostaglandin levels are also lowered by ethanol while the release of histamine, influx of calcium ions, generation of free radicals and production of leukotriens are all increased (Glavin and Szabo, 1992). The products of the 5-lipoxygenase pathway may also play a key role in the development of ulcer-induced by irritant agents such as ethanol. *V. hastata* protected the gastric mucosa of rats against ethanol-induced acute mucosal damage with a reduction of ulcer index indicating that the plant extract could be an effective gastroprotective agent. The mechanism for the mucosal protective action of the extract may be due partly to the stimulation of prostaglandin synthesis since endogenous prostaglandins play an important role in gastrocytoprotection.

The active constituents of the leaf extract (especially the tannins) may also have a contributory role to play in its anti-ulcer activity. Tannins are known to 'tar' the outermost layer of the gastric mucosa rendering it less permeable and more resistant to chemical and mechanical injury or irritant (Asuzu and Onu, 1990). It is possible that the flavonoids present in *V. hastata* may also play a role in this regard. Flavonoids possess anti-oxidant properties in addition to strengthening the mucosal defence system through stimulation of gastric mucus secretion (Martin et al., 1994). However, flavonoids can scavenge for the reactive oxygen species (e.g. super-oxide anions) and free radicals produced by ethanol. These reactive intermediates are potentially implicated in ulcerogenicity (Lewis and Hanson, 1991).

The leaf extract of *V. hastata* exhibited no antimicrobial activity against all test organisms used in the study. However, this was not un-expected since different parts of the same plant may have different properties and also show different activities. The acute toxicity profile of this plant extract could also be considered favourable judging from the high oral LD₅₀ value obtained and the absence of adverse clinical manifestations (e.g. respiratory distress, uncoordinated muscle movement etc.) in mice after 4 days observation.

In conclusion, the result of the present study clearly indicates that *V. hastata* is a potential therapeutic option in the effective management of ulcer, thus justifying its folkloric use by the local population for this purpose. However, there is a need for further studies in order to confirm these results with more details.

ACKNOWLEDGEMENTS

The authors are grateful to Obinna Okwuosa and Ifediniru Chikeluba for their assistance

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