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***Verbena bonnariensis* protects the gastric mucosa of gastric ulcer in rats**

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ABSTRACT

Verbena bonnariensis is concerned in sustaining the gastric mucosal integrity in gastric ulcer disease. However, the role of *V. bonnariensis* is not clear in this activity. The present study was designed to explore the effects of *V. bonnariensis* on gastric mucosal damage following gastric ulcer disease. In this study, the gastroprotective mechanism of *V. bonnariensis* was investigated. The extract and fractions of this plant were evaluated against cold restraint (CRU), aspirin (AS), alcohol (AL) and pyloric ligation (PL) induced gastric ulcer models in rats. Potential anti-ulcer activity of *V. bonnariensis* was observed against CRU (50.00%), AS (37.50%), AL (69.50%), and PL (50.00%) induced ulcer models. The standard drug omeprazole (10mg/kg, p.o.) showed 77.34% protection against CRU, 58.50% against AS and 69.42% against PL induced ulcer model. Sucralfate, another standard drug (500 mg/kg, p.o.) showed 62.50% protection in AL induced ulcer model. It significantly reduced free acidity (23.15%), total acidity (10.59%) and upregulated mucin secretion by 40.86% respectively. Conclusively, *V. bonnariensis* was found to possess anti-ulcerogenic activity which might be due to its anti-secretory activity and subsequent strengthening of the defensive mechanism. This study is the first of its kind to show significant anti-ulcer effect of *V. bonnariensis*. Therefore it could act as a potent therapeutic agent against peptic ulcer disease.

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KEYWORDS

Gastric ulcer;
Gastric acid secretion;
V. bonnariensis;
Medicinal plant.

INTRODUCTION

The major breakthrough in the search for alternative approaches for Gastric ulcer disease (GUD) treatment is the discovery of the protective influences of *Verbena* in GUD. Gastric ulcer disease affects a large population of the world. Which occur due to an imbalance between offensive (acid, pepsin and *Helicobacter pylori*) and defensive (mucin, prostaglandin and bicar-

bonate) factors. Consequently reduction of gastric acid production as well as reinforcement of gastric mucosal protection has been the major therapeutic approaches of peptic ulcer disease^[1]. A number of anti-ulcer drugs including proton pump inhibitors (PPI) and H₂ receptor antagonists are available for the treatment of GUD, but clinical evaluation of these drugs has shown incidence of relapse, side effects and drug interactions. This has been the rationale for the development of new anti-ul-

cer drugs and thus the search for novel molecules has been extended to medicinal plants that can offer better protection and decrease relapses.

Several Indian medicinal plant species like *Allophylus serratus*^[4], *Desmodium gangeticum*^[5], *Ocimum sanctum*^[6], *Xylocarpus granatum*, etc. have been reported to possess anti-ulcer activity. Studies on different biological activities of *C. roseus* in general are also available. But there is less information available regarding its pharmacological effect on the gastrointestinal system. Keeping these facts in considerations, we have assessed the anti-ulcer activity of the plant species, *Verbena bonnariensis*.

The purpose of the present study, however is to evaluate the anti-ulcerogenic effect of the *V. bonnariensis* against different experimental gastric ulcer models in rats and also to identify the active constituents responsible for these gastroprotective effects.

MATERIALS AND METHODS

Plant material

Plant was collected from the Indian gardens and was authenticated by the botany division of the Institute. The voucher specimen of the plant has been preserved in the herbarium of the Botany division with the code number 1766.

Extraction/fractionation procedure

The shade dried leaves (500 g) of the plant were powdered and extracted with 95% ethanol four times (4x1.0 lit). Combined extract was filtered and concentrated under reduced pressure to a green viscous mass. It was again dried under high vacuum to remove last traces of the solvent (21.5 g). This ethanolic extract has been used for antiulcer activity.

Experimental animals

All experimental protocols were approved by our Institutional Ethical Committee following the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) which complies with International norms of INSA (Indian National Science Academy). Adult Sprague Dawley rats of either sex, weighing 180-200g were housed in raised bottom mesh cages to prevent coprophagy and were

kept in environmentally controlled rooms ($25 \pm 2^\circ\text{C}$, 12 hours light and dark cycle). Animals were fed with standard laboratory food pellets and water was provided *ad libitum*.

Materials

Sucralfate was obtained from Meranani Pharmaceuticals, India, whereas omeprazole and other chemicals were obtained from M/s. Sigma Chemicals, St Louis, MO, USA.

Treatment schedule

Ethanol extract of *V. bonnariensis* (100 mg/kg) and omeprazole (Omz) (10 mg/kg) and sucralfate (SUC) (500 mg/kg) were prepared in 1% carboxymethyl cellulose (CMC) as suspension and administered orally 45 mins prior to exposure of ulcerogens to the animals at a volume of 1ml/200g of body weight. All animals were deprived of food for 16 h before ulcerogens exposure and were divided into three groups, (n=6).

1. Control group of animals were treated with vehicle 1% CMC.
2. Graded doses of ethanol extract of the plant (50, 100 and 200 mg/kg, p.o.) were tested against Cold restraint ulcer (CRU) model to identify the effective dose and selected for further studies in other ulcer models.
3. Experimental group was treated with standard anti-ulcer drugs such as Omz (10 mg/kg, p.o.) in (CRU), aspirin (AS), pyloric ligation (PL) and SUC (500 mg/kg, p.o.) in Alcohol (AL) induced ulcer model.

Anti-ulcer studies

(a) Cold restraint induced gastric ulcer (CRU)

Animals were subjected to cold restraint stress after 45 mins of treatment with *Verbena* (100 mg/kg) and Omz. All the animals were immobilized in restraint cage and kept at 4°C in an environmental chamber^[16]. Two hours later the animals were sacrificed and stomachs were observed and scored under Magnascope for ulcers.

(b) Aspirin induced gastric ulcer model (AS)

Aspirin at a dose of 150 mg/kg was administered to induce ulcer after 45mins of treatment of ethanol extract of the plant and Omz. The animals were sacrificed 5 hours after aspirin treatment^[10] and the stomach was

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dissected out, incised along the lesser curvature and the lesion was scored.

(c) Alcohol induced gastric ulcers in rats (AL)

Gastric ulcer was induced in rats by administering chilled absolute alcohol (1ml/200g, body weight of animals)^[16]. The *V. bonnariensis* and sucralfate were administered 45 minutes before alcohol treatment. After 1 hour of alcohol administration, the animals were sacrificed and stomach was cut open along the greater curvature to observe the gastric lesions which appear as hemorrhagic bands along the mucosal ridges of the stomach. The lengths of the lesions were measured using Biovis image analyzer software and summated to give a total lesion score.

(d) Pyloric ligation induced ulcer model (PL)

After 45 min. of administration of the ethanol extract of the plant and Omz, ulcer was induced in rats by pyloric ligation. Under Chloral hydrate anesthesia (300mg/kg, i.p.), the abdomen was opened and the pyloric end of the stomach was ligated avoiding any damage to the adjacent blood vessels^[14]. Stomach was replaced carefully and the animals were allowed to recover with free access to water. After 4 hours the animals were sacrificed and the stomach was dissected out. Lesions were scored and gastric fluid was collected and centrifuged at 2000 rpm for 10 min. The collected supernatant was used for the estimation of gastric secretion studies, mucin estimation and peptic activity.

Gastric secretion study

Free and total acidity was measured from the collected gastric juice by titrating against 0.01N NaOH, using phenolphthalein as an indicator and expressed in terms of $\mu\text{equiv./ml}$ ^[1]. Mucus content was expressed in terms of mg%^[18].

Measurement of ulcer index

Ulcer formed due to treatment with different ulcerogens were observed under Magnascope (5X magnification) and were scored according to the arbitrary scoring system as described by Srivastava et al.,^[15]. The severity and intensity of the lesions were graded as following: i) Shedding of epithelium = 10; (ii) Petechial and frank hemorrhages = 20; (iii) one or two ulcers = 30; (iv) more than two ulcers = 40; and (v) Perforated

ulcers = 50.

Statistical analysis

All values shown in the figures and tables represent the means \pm S.E.M. values with 95% confidence limits were estimated using Maximum Likelihood Iterative Procedure^[8]. Statistical analysis was performed with Prism version 3.0 software using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. $P < 0.05$ was considered to be statistically significant.

RESULTS

Anti-ulcer effect of *Verbena* against cold restraint and alcohol induced ulcer in rats

In our preliminary study, graded doses of the ethanol extract (50, 100 and 200mg/kg, p.o.) showed percentage protection of 25.0, 50.0 and 58.5 ($P < 0.05$) respectively whereas standard drug, omeprazole showed a percentage protection of 77.34 ($P < 0.01$) in comparison to control against CRU model Figure 1.

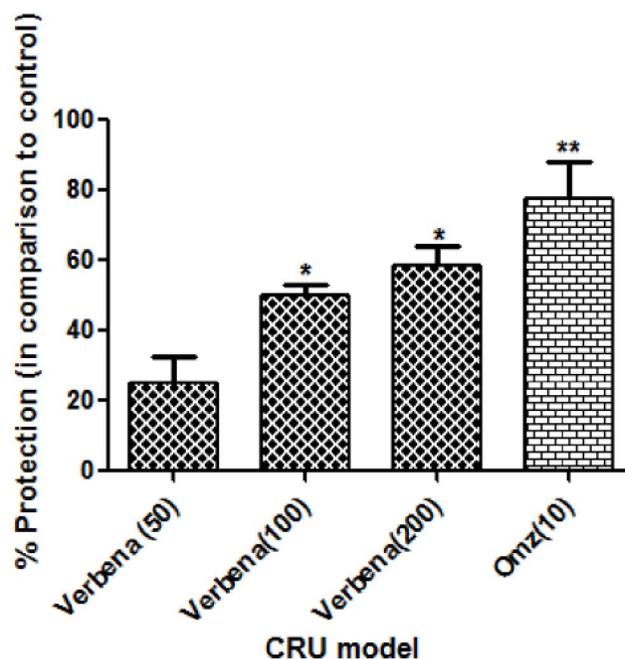


Figure 1 : Effect of the ethanol extract of *V.bonnariensis* and standard drugs (Omz) on percentage protection of ulcer against cold restraint induced gastric ulcer models in rats. Data expressed as mean % protection \pm S.E.M. Statistical analysis was done by One Way ANOVA followed by dunnett's multiple comparison test. *Statistically significant at $P < 0.05$ and ** $P < 0.01$, in comparison to control. n = 6 in each group.

Effect of the ethanol extract of *V. bonnariensis* against aspirin induced ulcer in rats

Anti-ulcer activity of ethanol extract of the plant (100 mg/kg) was observed when its efficacy was tested against aspirin induced ulcer model 37.5% protection ($P < 0.01$) was observed when the ethanol extract was administered whereas omeprazole showed 58.50% protection in comparison to control as shown in Figure 2.

Effect of *Verbena* against alcohol induced ulcer

In anti-ulcer activity against ethanol induced ulcer, of ethanol extract of the plant (100 mg/kg) showed significant having 69.5% protection ($P < 0.01$), whereas the standard drug, sucralfate, showed 62.50% protection ($P < 0.05$) as depicted in Figure 2.

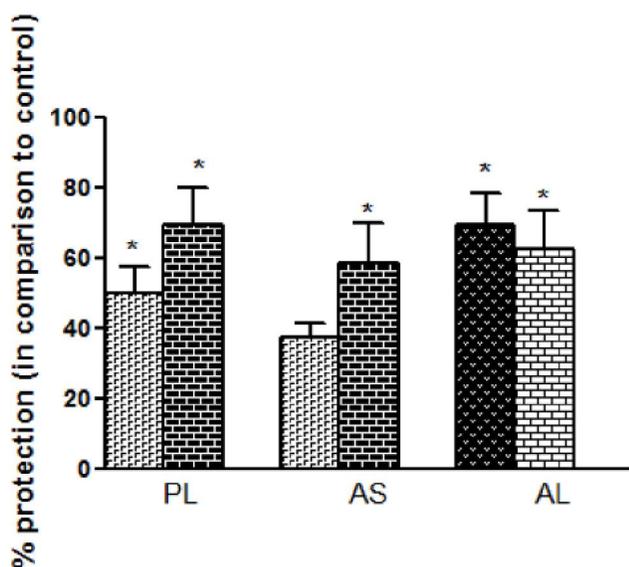


Figure 2 : Effect of the ethanol extract of *V. bonnariensis* and standard drugs (Omz and SUC) on percentage protection of ulcer against cold restraint, Aspirin, pyloric ligation and alcohol induced gastric ulcer models in rats. Data expressed as mean % protection \pm S.E.M. Statistical analysis was done by One Way ANOVA followed by dunnett's multiple comparison test. *Statistically significant at $P < 0.05$ and ** $P < 0.01$, in comparison to control. n = 6 in each group.

Anti-ulcer effect of *Verbena* against pyloric ligation induced ulcer in rats

Anti-ulcer activity of the ethanol extract of the plant was also observed against pyloric ligation induced ulcer in rats where it showed protection of 50.0% ($P < 0.01$) and omeprazole showed 77.73% ($P < 0.01$) protection (Figure 2).

Effect of *Verbena* on gastric secretion

The antisecretory effect of the ethanol extract of the plant was evaluated by estimating free and total acidity of gastric juice and mucin as shown in TABLE 1. The extract has reduced free acidity (23.15%), total acidity (10.59%) which was comparable with standard drug omeprazole (61.88%, $P < 0.001$) and (38.43%, $P < 0.01$) respectively. It significantly upregulated mucin secretion by 40.86% ($P < 0.05$) whereas omeprazole increased mucin secretion by 33.13% ($P < 0.05$) in comparison to control.

TABLE 1 : Effect of the ethanol extract of *V. bonnariensis* and omeprazole on free acidity, total acidity and mucin contents in pyloric ligation model (n= 6 in each group).

Treatment	Free acid μ equiv./ml	Total acid μ equiv./ml	Mucin μ g/ml
Control	38.91 \pm 8.036	88.29 \pm 6.537	544.91 \pm 249.3
Verbena (100mg/kg)	29.90 \pm 11.265	78.94 \pm 8.001	921.49 \pm 302.8**
Omz(10mg/kg)	14.83 \pm 2.714**	54.36 \pm 3.594**	814.97 \pm 106.2*

DISCUSSION

Regulation of inflammatory response is an essential element in the pathogenesis of a variety of inflammatory disorders including gastric ulcer. Amelioration of these inflammatory events might accelerate healing of gastric ulcer. Thus, we hypothesized *V. Bonnariensis* extract might promote resolution of inflammation during protection against gastric ulcer. In our modern times, the use of therapeutic plants and natural products has become universal. The discovery of new and novel pharmaceutical products from plants used in traditional system of medicine or folklore for the treatment or amelioration of the incidence of gastric ulcers^[2]. The anti-ulcer activity of the ethanolic extract of the *V. bonnariensis* has been studied against various models of experimentally induced gastric ulcer in order to evaluate its mechanism of action involved in prevention of ulcer formation. The finding receives an impetus by considering the fact that the ethanolic extract of the *V. bonnariensis* showed anti-ulcerogenic activity in all the models, each of which induced ulcer through a different mechanism.

Gastric ulcer is postulated to develop when there is a disbalance of aggressive and defensive factors either because of increased secretion of acid or pepsin or because of impairment of mucosal resistance. So we

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select two models one of antisecretory and other of cytoprotective for the preliminary gastroprotective study of the ethanol extract of the *V. bonnariensis*.

We performed a dose dependent anti-ulcer study of the ethanolic extract of the *V. bonnariensis* in CRU model. CRU is a well-accepted model for the induction of gastric ulcers, in which peripheral sympathetic activation and increased acid secretion play important roles^[7]. The ethanolic extract of the *V. bonnariensis* exhibited significant protection in a dose dependent manner in the CRU model, with maximum protection observed at 100mg/kg, p.o. In addition, it exerted a protective effect against ethanol-induced gastric lesions in contrast to standard drug, sucralfate. Since ethanol damages the superficial epithelial layers and inhibit the release of mucosal prostaglandins^[13] and depresses the gastric defensive mechanisms, these agents appear to augment the gastric mucosal defense^[12] indicating the cytoprotective potentials of the ethanolic extract of the *V. bonnariensis*.

The ethanolic extract of the *V. bonnariensis* was effective in decreasing the hemorrhagic lesions induced by ethanol in contrast to standard drug, sucralfate, reflecting its cytoprotective activity.

Furthermore, gastric acid is an important factor for the genesis of ulceration in pyloric-ligated model^[14]. In this model, auto-digestion of mucosa by gastric acid and pepsin results in the development of ulcers^[9]. *Verbena* reduced free and total acidity in this model, which suggests its anti-secretory potency.

The cytoprotective ability of the ethanolic extract of the *V. bonnariensis* was evident with increase in mucin content in pyloric ligation model and protection against ethanol induced ulcer model in comparison with the standard drugs. To further substantiate the cytoprotective potency of the ethanolic extract of the *V. bonnariensis*, its effect against NSAIDs induced ulcer model was explored. Studies suggest that NSAIDs induces ulcers due to their effect on cyclooxygenase enzyme leading to reduced prostaglandin production and increase in acid secretion^[3,9]. The ethanolic extract of the *V. bonnariensis* significantly reduced ulcer incidence, which further supports cytoprotective effect of the ethanolic extract of the *V. bonnariensis*, which may be mediated by prostaglandins.

Different biological activities of the plant

V. bonnariensis has been reported earlier, anti-ulcer activity of this plant has not been reported till date. Our study is the first of its kind to show significant anti-ulcer effect of *V. bonnariensis*. Thus, the present study demonstrated that the *V. bonnariensis* impart gastroprotective effects. Thus, *V. bonnariensis* may emerge as a more potent therapeutic agent in treating gastric ulcer incidences since *V. bonnariensis* possess both anti-secretory and cytoprotective potentials.

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Full Paper

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