Vincamine and Vindoline from *Catharanthus roseus* linn. Protects the Gastric Mucosa of Gastric Ulcer in Rats

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**ABSTRACT**

**Background:** Currently, natural products have been shown to present interesting biological and pharmacological activities and are used as chemotherapeutic agents. Plants have historically been used in treating cancer and are recognized for their ability to produce secondary metabolites. **Objective:** The current study was designed to evaluate the antiulcer activity of total extract as well as several fractions from *Catharanthus roseus* Linn. (Family: Apocynaceae) leaves. The bioassay guided chloroform fraction of the ethanol extract yielded two major compounds which have shown a promising antiulcer activity. **Materials and methods:** *C. roseus* leaves were evaluated against Cold Restraint Ulcer (CRU), Aspirin (AS), Alcohol (AL) and Pyloric ligation (PL) induced gastric ulcer models in rats. Potential anti-ulcer activity was observed. **Results:** Potential anti-ulcer activity was observed against CRU (75.18%), AS (50.00%), AL (65.00%) and PL (50.00%) induced ulcer models. The standard drug omeprazole (10 mg kg⁻¹, p.o.) showed 77.34% protection against CRU, 57.08% against AS and 69.42% against PL induced ulcer model. Sucralfate, another standard drug (500 mg kg⁻¹, p.o.) showed 62.72% protection in AL induced ulcer model. Ethanolic extract of *C. roseus* leaves significantly reduced free acidity (17.78%), total acidity (8.05%) and up regulated mucin secretion by 25.11%, respectively. Phytochemical investigations of chloroform fraction yielded vincamine and vindoline. Further, Fr-CHCl₃ and its compounds vincamine and vindoline significantly showing protection against CRU 81.08 and 81.20%, respectively, confirming their anti-ulcer activity. **Discussion and conclusion:** The anti-ulcerogenic activity of the chloroform fraction might be due to its anti-secretory activity. This study is the first of its kind to show significant anti-ulcer effect of *C. roseus*. Therefore, it could act as a potent therapeutic agent against peptic ulcer disease.

**Key words:** Gastric ulcer, gastric acid secretion, *Catharanthus roseus*, medicinal plant


**INTRODUCTION**

Peptic ulcer disease affects a large population of the world, it occur due to an imbalance between offensive (acid, pepsin and *Helicobacter pylori*) and defensive (mucin, prostaglandin and bicarbonate) factors. Consequently reduction of gastric acid production as well as reinforcement of gastric mucosal protection has been the major therapeutic approaches of peptic ulcer disease¹. A number of anti-ulcer drugs including Proton Pump Inhibitors (PPI) and H₂ receptor antagonists are available for the treatment of PUD 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-ulcer 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 *Allophyllus serratus* Roxb.¹, *Desmodium gangeticum*⁷, *Oxylum sanctum* L.², *Xylocarpus granatum* König⁴, *Asparagus raemulosus*⁵ 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 *C. roseus*.

The purpose of the present study however, was to evaluate the anti-ulcerogenic effect of the *C. roseus* leaves and its chloroform fraction against different gastric ulcer models in rats and to identify the active constituents responsible for the gastro protective effects.

**MATERIALS AND METHODS**

**Collection of the plant:** The leaves of *Catharanthus roseus* Linn. were collected from Lucknow gardens in the month of March 2009 and were
authenticated by the Botany Division of The Central Drug Research Institute, Lucknow and has been preserved in the herbarium of the Institute.

**Extraction, fractionation and isolation procedure:** Shade dried powdered leaves (2.0 kg) were extracted with 95% ethanol at room temperature. The extract was filtered after 24 h and fresh ethanol was added, the process was repeated 4 times and the combined filtered ethanol extract was concentrated in a rotatory evaporator below 50°C. The green viscous mass thus obtained was further dried under high vacuum to remove last traces of solvent (wt. 42.5 g). The ethanol extract (40.8 g) was fractionated with hexane (5.1 g), chloroform (10.5 g) and n-butanol (4.5 g) soluble fractions successively by maceration and n-butanol insoluble fraction (19.8 g). The chloroform fraction showing potent antulcer activity was purified by repeated column chromatography. Two major compounds, vincamine⁶ and vindoline⁷ (Fig. 1. 2) were obtained which were identified by their physicochemical data and Co-TLC with authentic samples.

**Animals:** Adult Sprague Dawley rats of either sex, weighing 180-200 g were housed in raised bottom mesh cages to prevent coprophagy and were kept in environmentally controlled rooms (25±2°C, 12 h light and dark cycle). Animals were fed with standard laboratory food pellets and water was provided as ad libitum. Guinea pigs of either sex, weighing 300-350 g were used for histamine-induced ulcer model which were also housed under standard conditions as described above. 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). Sucralfate was obtained from Meranani Pharmaceuticals, India, whereas omeprazole and other chemicals were obtained from Ms. Sigma Chemicals, St Louis, MO, USA.

**Treatment schedule:** The ethanol extract of *C. roseus* leaves and its chloroform fraction and hexane fraction, omeprazole (Omz) (10 mg kg⁻¹) and sucralfate (SUC) (500 mg kg⁻¹) were prepared in 1% carboxymethyl cellulose (CMC) as suspension and 45 min prior to exposure of ulcerogens was administered orally to the animals at a volume of 1 mL⁻¹ 200 g of b. wt. All the animals were deprived of food for 16 h before ulcerogens exposure and were divided into three groups (n = 6):

- Control group of animals were treated with vehicle 1% CMC
- Graded doses of ethanol extract of *C. roseus* leaves (100 and 200 mg kg⁻¹, p.o.) and its chloroform fraction (10, 20 and 40 mg kg⁻¹, p.o.) were tested against Cold Restraint Ulcer (CRU) model to identify the effective dose and selected for further studies in other ulcer models
- Experimental group was treated with standard anti-ulcer drugs such as Omz (10 mg kg⁻¹, p.o.) in (CRU), aspirin (AS), pyloric ligation (PL) and SUC (500 mg kg⁻¹, p.o.) in alcohol (AL) induced ulcer model
ANTULCER STUDIES
Cold restraint induced gastric ulcer (CRU): Animals were subjected to cold restraint stress after 45 min of treatment with ethanol extract, hexane, chloroform fractions and Omz. All the animals were immobilized in restraint cage and kept at 4°C in an environmental chamber. Two hours later the animals were sacrificed and stomachs were observed and scored under magnascope for ulcers.

Aspirin induced gastric ulcer model (AS): Aspirin was administered at a dose of 150 mg kg⁻¹ to induce ulcer, after 45 min. of treatment of ethanol extract and Omz. The animals were sacrificed after 5 h of aspirin treatment and the stomachs were dissected out, incised along the lesser curvature and the lesion was scored.

Alcohol induced gastric ulcers in rats (AL): Gastric ulcer was induced in rats by administering chilled absolute alcohol (1 mL 200 g⁻¹, b. wt. of animals). The ethanol extract and sulfate were administered 45 min before alcohol treatment. After 1 h of alcohol administration, the animals were sacrificed and stomachs were cut open along the greater curvature to observe the gastric lesions which appear as hemorrhagic bands along the mucosal ridges of the stomachs. The lengths of the lesions were measured using Biovis image analyzer software and summated to give a total lesion score.

Pyloric ligation induced ulcer model (PL): After 45 min of administration of E-EtOH and Omz, ulcer was induced in rats by pyloric ligation. Under chloral hydrate anesthesia (300 mg kg⁻¹, i.p.), the abdomen was opened and the pyloric end of the stomach was ligated avoiding any damage to the adjacent blood vessels. Stomach was replaced carefully and the animals were allowed to recover with free access to water. After 4 h 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, mucus 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 meq mL⁻¹. Peptic activity was determined by measuring the amount of liberated tyrosine by the action of pepsin on hemoglobin as substrate and expressed in terms of units mL⁻¹. Mucus content was expressed in terms of percentage.

Measurement of ulcer index: Ulcers formed due to treatment with different ulcerogens were observed under magnascope (5X magnification) and were scored according to the arbitrary scoring system. The severity and intensity of the lesions were graded as following:

- Shedding of epithelium = 10
- Petechial and frank hemorrhages = 20
- One or two ulcers = 30
- More than two ulcers = 40
- Perforated ulcers = 50

Table 1: Effect of Fr-Hex, vinsamine, vindoline and standard drug omeprazole on percentage protection against cold restraint induced gastric ulcer in rats

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Percentage protection in cold restraint ulcer model (mg kg⁻¹; p.o.)</th>
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<tbody>
<tr>
<td>Fr-Hex</td>
<td>0.00 24.5 25.0</td>
</tr>
<tr>
<td>Vinsamine</td>
<td>50.0 81.2** 80.0**</td>
</tr>
<tr>
<td>Vindoline</td>
<td>42.5 81.2** 78.5**</td>
</tr>
<tr>
<td>Omeprazole 10</td>
<td>77.4**</td>
</tr>
</tbody>
</table>

*Statistically significant at p<0.05, **p<0.01 and ***p<0.001, in comparison to control, n-6 in each group, values expressed as percentage of protection ± SEM.

Table 2: Effect of CR and Omeprazole on free acidity, total acidity and mucin contents in pyloric ligation model (n-6 in each group)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Free acid (meq mL⁻¹)</th>
<th>Total acid (meq mL⁻¹)</th>
<th>Mucin (μg mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>65.00±9.00</td>
<td>108.00±12.00</td>
<td>5780.00±13.76</td>
</tr>
<tr>
<td>CR (100 mg kg⁻¹)</td>
<td>51.80±1.09</td>
<td>99.30±4.48</td>
<td>5047.50±9.23</td>
</tr>
<tr>
<td>Omz (10 mg kg⁻¹)</td>
<td>35.00±2.89</td>
<td>58.30±1.16</td>
<td>4892.50±9.91</td>
</tr>
</tbody>
</table>

*Statistically significant at p<0.05 and **p<0.01, in comparison to control, n-6 in each group.

Fig. 3: Effect of E-EtOH of Catharanthus roseus and standard drugs (Omz and SUC) on percentage protection of ulcer against cold restraint and alcohol induced gastric ulcer models in rats. Data expressed as mean percentage protection ± SEM. *Statistically significant at p<0.05 and **p<0.01, in comparison to control, n-6 in each group.
RESULTS AND DISCUSSION

Anti-ulcer effect of ethanol extract against cold restraint and alcohol induced ulcer models in rats: In our preliminary study, graded doses of ethanol extract (100 and 200 mg kg⁻¹, p.o.) of C. roseus showed percentage protection of 75.18 and 74.32 (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. In addition, ethanol extract (100 mg kg⁻¹, p.o.) showed 65.0% protection against alcohol induced gastric ulcer model, whereas standard drug sucralfate showed 64.72% protection (Fig. 3).

Anti-ulcer effect of Fr-CHCl₃ and Fr-Hex and its active constituents vinamine and vindoline against cold restraint induced ulcer in rats: Graded doses of chloroform fraction (10, 20 and 40 mg kg⁻¹, p.o.) showed percentage protection of 20.5, 45.83 (p<0.01) and 37.0 (p<0.01), respectively. Hexane fraction (10, 20 and 40 mg kg⁻¹, p.o.) showed percentage protection of 0.0, 24.5 (p<0.01) and 25.0 (p<0.01), respectively, whereas standard drug, omeprazole showed a percentage protection of 77.34 (p<0.01) in comparison to control against CRU model. From this observation 20 mg kg⁻¹ dose of chloroform fraction was identified as the effective dose and selected for further studies. The results are graphically represented in Fig. 5 and Table 1. Effect of ethanol extract against aspirin induced ulcer in rats.

Potential anti-ulcer activity of ethanol extract was observed when its efficacy was tested against aspirin induced ulcer model 50.0% protection (p<0.01) was observed when ethanol extract was administered whereas omeprazole showed 57.08% protection in comparison to control as shown in Fig. 3.

Effect of ethanol extract against alcohol induced ulcer: Ethanol extract showed significant anti-ulcer activity against ethanol induced ulcer having 65.0% protection (p<0.01), whereas the standard drug, sucralfate, showed 62.55% protection (p<0.05) as depicted in Fig. 3.

Anti-ulcer effect of ethanol extract against pyloric ligation induced ulcer in rats: Anti-ulcer activity of ethanol extract 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 (Fig. 3).
Effect of ethanol extract on gastric secretion: The antiseretary effect of ethanol extract was evaluated by estimating free and total acidity of gastric juice and by estimating the activity of pepsin and mucin as shown in Table 2. Ethanol extract has reduced free acidity (17.78%), total acidity (8.05%) which was comparable with standard drug omeprazole (44.44%, p<0.001) and (46.01%, p<0.01), respectively. It significantly up regulated mucin secretion by 25.11% (p<0.05) whereas omeprazole increased mucin secretion by 22.73% (p<0.05) in comparison to control. In our contemporary times, the use of medicinal 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 ulcers15. The anti-ulcer activity of ethanol extract of C. roseus leaves 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 ethanol extract of C. roseus showed anti-ulcerogenic activity in all the models, each of which induced ulcer through a different mechanism. Peptic 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 select two models one of antisecretory and other of cytoprotective for the preliminary gastroprotective study of ethanol extract of C. roseus leaves. We performed a dose dependent anti-ulcer study of ethanol extract 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 roles15. Ethanol extract exhibited significant protection in a dose dependent manner in the CRU model, with maximum protection observed at 100 mg kg⁻¹, p.o. In addition, ethanol extract 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 prostaglandins15 and depresses the gastric defensive mechanisms, these agents appear to augment the gastric mucosal defense15 indicating the cytoprotective potentials of ethanol extract of C. roseus leaves. For the further identification of the phytochemical constituents responsible for the above mentioned anti-ulcer effect, ethanol extract was fractionated into chloroform fraction and tested in CRU gastric ulcer models. Graded doses of chloroform fraction exerted anti-ulcer effect in the CRU model, offering maximum protection at much lower dose 20 mg kg⁻¹ than ethanol extract (100 mg kg⁻¹) indicating concentration of active constituents in this fraction. Hence, 20 mg kg⁻¹ dose was considered to be the optimal dose for evaluation in further studies. Chloroform fraction 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 model15. In this model, auto-digestion of mucosa by gastric acid and pepsin results in the development of ulcers21. Ethanol extract reduced free and total acidity in this model which suggests its anti-secretory potency. The cytoprotective ability of ethanol extract, 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 ethanol extract, its effect against NSAIDs induced ulcer model was explored. Studies suggest that NSAIDs induce ulcers due to their effect on cyclooxygenase enzyme leading to reduced prostaglandin production and increase in acid secretion21,22. Ethanol extract significantly reduced ulcer incidence which further supports cytoprotective effect of ethanol extract which may be mediated by prostaglandins. Phytochemical investigations of the chloroform fraction demonstrated the presence of pure compounds namely vincamine and vindoline. The gastroprotective activity of the compounds vincamine and vindoline is not well established. Thus, we investigated the effect of vincamine and vindoline on CRU. Vincamine and vindoline inhibited the gastric ulceration and signifying that anti-ulcer activity of the C. roseus might be attributed to the presence of these compounds. Though different biological activities of the plant C. roseus 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 vincamine and vindoline isolated from the chloroform fraction of C. roseus leaves.

CONCLUSION

Thus, the present study demonstrated that the chloroform fraction of C. roseus and its active constituents vincamine and vindoline impart gastroprotective effects. The pure compounds (vincamine and vindoline) isolated from the active fraction fraction of C. roseus may emerge as a more potent therapeutic agent in treating gastric ulcer incidences since C. roseus possess both anti-secretory and cytoprotective potentials.

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