Antiulcer potential of leaves of achyranthes aspera Linn. in rats

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ABSTRACT

Plants have been used for medicine from time immemorial because they have fitted the immediate personal need, are easily accessible and inexpensive. In the recent past there has been a tremendous increase in the use of plant based health products in developing as well as developed countries resulting in an exponential growth of herbal products globally. Herbal medicines have a strong traditional or conceptual base and the potential to be useful as drugs in terms of safety and effectiveness leads for treating different diseases. No studies have been reported for its antiulcer activity. Therefore, an attempt has been made to evaluate the antiulcer potential of the ethanolic extract of Achyranthes aspera Linn.. It was found that ethanolic extract of the leaves of A.aspera was active in various ulcer models in Sprague-dawley (SD) rats. It showed gastroprotection by following the antisecretory and cytoprotective mechanism. In anti secretory mechanism the ethanolic extract of the A.aspera was inhibiting the free and total acid in pyloric ligated ulcer models. It was showing protection in alcohol induced ulcer model and it also improved the depleted levels of mucin in pyloric ligated model.

INTRODUCTION

Plants have been part of our lives since the beginning of time. We get numerous products from plants, most of them not only good and beneficial but also crucial to our existence. Plants are the backbone of all life on earth and an essential resource for human well-being. Just think about how your everyday life depends on plants. Everything we eat comes directly or indirectly from plants. Throughout human history, approximately 7,000 different plant species have been used as food by people. Oxygen is brought to you by plants, as a byproduct of photosynthesis. One-quarter of all prescription drugs come directly from or are derivatives of plants.

Gastric ulcer is an attacked area of the gastric or duodenal mucosa caused by the action of gastric acid and decreased defensive factors. Due to lack of equilibrium between gastric aggressive (acid, pepsin, Helicobacter pylori and bile salts) and the mucosal defensive factors (mainly involve mucus, bicarbonate secretion and prostaglandins)⁴¹. Therefore the modern approach to control gastric ulceration is to inhibit gastric acid secretion and to promote gastroprotection for effective healing. Presently this is achieved through the use of Antacids, proton pump inhibitors, and histamine
H₂ receptor antagonists are commonly used drugs\[^{[2,3]}\]. However, beside their therapeutic efficacies, several incidences of relapse, adverse effects and drug interactions have been shown to be associated with these drugs\[^{[4]}\]. H₂-receptor antagonists or the irreversible H⁺K⁺ATPase inhibitors popularly referred to as proton pump inhibitors (PPIs). But they show drug interactions and incidence of relapses. In order to avoid these adverse effects, herbal molecules obtained from plant extracts are one of the preferable sources as they are proved to be safe, clinically effective and more economic.

Thus in order to overcome these drawbacks investigation has been extended for the search of new and novel molecules from plant sources which can show better protection and lesser rate of incidence of relapse. Hence, research has been focused on search for new anti-ulcer molecules from medicinal plants as these molecules are more relevant to living system and already have definite biological functions and thus, may have fewer side effects. As a part of anti-ulcer drug discovery programs of our lab several Indian medicinal plants including *Terminalia chebula*\[^{[5]}\] and *Xylocarpus granatum*\[^{[6]}\] have been reported to possess anti-ulcer activity.

**MATERIALS AND METHODS**

**Plant material**

Leaves of *Achyranthes aspera* Linn. (1.0 Kg.) were collected from Lucknow, India and was authenticated by Botany Department of the Lucknow University.

**Extraction fractionation and isolation of compounds**

The air dried leaves (1.0 Kg.) were powdered and percolated in 95% ethanol at room temperature for 24 hours, filtered and the process was repeated four times. All the extracts were mixed and filtered. Mixed ethanolic extract was concentrated under reduced pressure below 50°C in a rotavapour to a green viscous mass, which was dried under high vacuum for 2 hours to remove the last traces of the solvent. Weight of the dried ethanolic extract 21.5 g. which was used for the screening against different gastric ulcer models.

**Experimental animals**

Sprague Dawley rats of either sex, weighing 180-200g were housed in elevated floor mesh cages to prevent coprophagy and were kept in environmentally controlled rooms (temperature 25±2°C and 12 hours light and dark cycle rotation). 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).

**Drugs and chemicals**

All chemicals were obtained from Sigma (St. Louis, MD, USA). Except Sucralfate, it was obtained from Meranani Pharmaceuticals, India.

**Dose selection and its schedule**

The extract of *A. aspera* was given orally to rats at different doses (100, 200 and 400mg/kg, p.o., b.w.) as well as reference drugs omeprazole (Omz) (10 mg/kg) and sucrafate (SUC) (500 mg/kg) were prepared in 1% carboxymethyl cellulose (CMC) as suspension and administered orally at a dose of 1ml/20g of body weight, 45 min. prior to exposure of ulcerogens. Animals were fasted for 16 hrs. before ulcerogens exposure and were divided into three groups, (n=6).

**Group I (control)**

Control group of animals were treated with vehicle (1% CMC), 45 min. before to exposure of ulcerogens to the animals.

**Group II (A. aspera extract treated)**

Animals were treated with active dose of *A. aspera* (20 mg/kg, p.o.), 45 min before to the induction of gastric ulcer in all ulceration models.

**Group III (reference drug treated)**

Third group was sub categorizes into further sub-groups based on selection of models.

**EFFECT OF A.ASPERA IN GASTROPROTECTION**

**Cold restraint induced gastric ulcer (CRU)**

In cold restraint stress model, all the animals were
treated with extract of *A. aspera* for 45 min., before subjected to ulceration. For reference drug omeprazole (Omz), same procedure were followed In CRU all groups were immobilized in restraint cage and kept at 4°C in an environmental chamber[7]. After two hours, animals were sacrificed and stomachs were observed and scored under Magnascope for ulcers.

**Alcohol induced gastric ulcers model (AL)**

Chilled absolute alcohols were given orally to the rats (1ml/200g, body weight), for induction of gastric ulcer[8] *Acyranthes aspera* and sucralfate (SUC) were administered 45 minutes before alcohol treatment. After 1 hour of alcohol administration, animals were sacrificed and stomachs were cut open along the greater curvature to observe the gastric lesions appearing as hemorrhagic bands along the mucosal ridges of the stomach. Lengths of the lesions were measured using Biovis image analyzer software and summed to give a total lesion score.

**Aspirin induced gastric ulcer model (AS)**

Extract of the *A. aspera* and reference drug omeprazole (Omz) were administered 45 min. before the treatment of aspirin (150 mg/kg body weight). Animals were sacrificed after 5 hours of aspirin treatment and the stomachs were dissected out, incised along the lesser curvature and the lesions were scored[9].

**Pyloric ligation induced gastric ulcer model (PL)**

Extract of the *A. aspera* and omeprazole (Omz), given orally before 45 min. of ulcer induction. Pyloric ligation was done under chloral hydrate anesthesia (300mg/kg, i.p.)[10]. After 4 hrs., animals were sacrificed and stomachs were dissected out. Lesions were scored and gastric fluid was collected and used for estimation of gastric secretion and mucin level.

**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 µ equiv./ml[11]. Mucin level in gastric juice was quantified as per method reported earlier[12].

**Ulcer scoring**

Magnascope (5X magnification) were used for ulcer scoring after induction of ulcer via different ulcerogens. Ulcers were scored According to method reported earlier[13]. 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.

**Statistical analysis**

All values shown in the figures and tables represent the means ± S.E.M. Statistical analysis was performed with Prism version 5.0 software using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. P<0.05 was considered to be statistically significant (p<0.001 = ***, p<0.01 = **, p<0.05 = *)

**RESULTS**

**Effect of extract of the A.aspera on cold restraint induced ulcer model in rats**

Administration of the extract of the *A. aspera* at graded doses of 100, 200 and 400mg/kg, p.o. exhibited 24.5%, 50.0% and 52.5% protection respectively which indicated antiulcer potential of this compounds (Figure 1).

**Effect of extract of the A.aspera on alcohol induced ulcer model in rats**

The extract of the *A.aspera* showed significant antiulcer activity against ethanol induced ulcer showing 68.51% protection whereas the reference drug, sucralfate (SUC), showed 65.50% protection respectively which indicated antiulcer potential of this compounds (Figure 1).

**Effect of extract of the A.aspera on aspirin induced ulcer model in rats**

The extract of the *A. aspera* showed 48.5% protection in aspirin induced ulcer model, whereas omeprazole showed 37.5% protection in comparison to control as shown in Figure 2.

**Effect of extract of the A.aspera on pyloric ligation induced ulcer model in rats**

The extract of the *A. aspera* was also observed against pyloric ligation induced ulcer model in SD rats where it showed protection of 50.0% whereas refer-
Figure 1: Effect of graded dose of the extract of the *A. aspera* (AA) and reference drug omeprazole (Omz) on percentage protection of ulcer against cold restraint induced gastric ulcer models in rats. Data expressed as mean of % protection of AA and reference drugs after ulcer induction ± 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.

Reference drug omeprazole (Omz) showed 62.5% protection (Figure 2).

Effect of extract of the *A. aspera* on gastric secretion

As shown in TABLE 1 and Figure 4, treatment with the extract of the *A. aspera* at a dose of 200 mg/kg body weight significantly reduced the free acidity by 19.78% and total acidity by 15.23%. Reference drug omeprazole significantly reduced the free acidity by 57.36% and total acidity by 53.71%. On the other side, the extract of the *A. aspera* increased the mucin secretion by 51.39% in comparison to control (TABLE 1). Whereas reference drug omeprazole increased 47.43% in comparison to control.

Figure 2: Effect of the extract of the *A. aspera* (AA) and reference drugs Omeprazole and Sucralfate (Omz and SUC) on percentage protection of ulcer against alcohol, pyloric ligation and Aspirin induced gastric ulcer models in rats. 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.

Figure 3: Effect of the extract of the *A. aspera* (AA) and reference drugs Sucralfate (SUC) on percentage protection of ulcer against alcohol induced gastric ulcer models in rats. 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.
Natural products are used as medicine, since from the ancient times, India is one of the seventeen mega diverse nations and considered as endemism having various species of medicinal plants at several degree of taxonomic levels. The anti-ulcer activity of the extract of the

**DISCUSSION**

Natural products are used as medicine, since from

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Free acid µequiv./ml</th>
<th>Total acid µequiv./ml</th>
<th>Mucin µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>45.5±6.09</td>
<td>57.23±4.81</td>
<td>419.7±87.23</td>
</tr>
<tr>
<td><em>Ethanol extract of A. aspera (AA) (200 mg/kg)</em></td>
<td>36.50±7.41*</td>
<td>48.51±9.37</td>
<td>863.51±106.47**</td>
</tr>
<tr>
<td>Omeprazole (10mg/kg)</td>
<td>19.40±2.73**</td>
<td>26.49±2.58**</td>
<td>798.48±95.31*</td>
</tr>
</tbody>
</table>

**Figure 4**: Effect of the extract of the *A. aspera* (AA) and reference drugs Omeprazole (Omz) on free acidity against pyloric ligation induced gastric ulcer models in rats. (a) Effect of the extract of the *A. aspera* (AA) and reference drugs Omeprazole (Omz) on total acidity against pyloric ligation induced gastric ulcer models in rats. (b) Effect of the extract of the *A. aspera* (AA) and reference drugs Omeprazole (Omz) on mucin contents against pyloric ligation induced gastric ulcer models in rats. (c) 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.

**TABLE 1**: Effect of the extract of the *A. aspera* (AA) and omeprazole (Omz) on free acidity, total acidity and mucin contents in pyloric ligation model (n= 6 in each group)
A. aspera (AA) has been studied against various ulcer models in order to evaluate its mechanism of action involved in prevention of gastric ulcer.

We performed a dose dependent anti-ulcer study of the extract of the A. aspera (AA) in CRU model. The extract of the A. aspera (AA) exhibited significant protection in a dose dependent manner in the CRU model, with maximum protection observed at 200mg/kg, p.o. CRU is a well-accepted model for the induction of gastric ulcers, in which peripheral sympathetic activation and increased acid secretion play important roles[14].

To check the antisecretory ability of the extract of the A. aspera (AA) we perform the pyloric ligation model. In pyloric-ligated model gastric acid is an important factor for the genesis of ulceration [10]. In this model, auto-digestion of mucosa by gastric acid results in the development of ulcers [15]. A. aspera (AA) significantly reduced free and total acidity in this model, which suggests its anti-secretory potency.

The extract of the A. aspera (AA) exerted a protective effect against ethanol-induced gastric lesions. Ethanol damages the superficial epithelial layers and inhibits the release of mucosal prostaglandins and depresses the gastric defensive mechanisms [16]. The extract of the A. aspera (AA) appear to support the gastric mucosal defense indicating the cytoprotective potentials.

Furthermore, to shore up the cytoprotective ability of the extract of the A. aspera (AA) we check the mucin level in pyloric ligation model and protection against ethanol induced ulcer model in comparison with the reference drugs. To further substantiate the cytoprotective potency of the extract of the A. aspera (AA), its effect against NSAIDs induced ulcer model was explored. Studies suggest that NSAIDs induce ulcers through their effect on cyclooxygenase enzyme leading to reduced prostaglandin production and increase in acid secretion [15,17]. Our result demonstrated that the extract of the A. aspera (AA) significantly increased gastric mucin level and exerted significant protection in ethanol and aspirin induced gastric lesions in rats.

Though different biological activities of the extract of the A. aspera (AA) have been reported earlier but anti-ulcer mechanism of the extract of the A. aspera (AA) has not been reported. Our study is the first of its kind to establish the anti-ulcer potential of A. aspera (AA).

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REFERENCES

[8] A.Robert; Cytoprotection by prostaglandins,


