A STUDY ON ANTI-ULCER ACTIVITY OF STEM EXTRACTS OF CUSCUTA REFLEXA (ROXB) AGAINST PYLORUS LIGATION INDUCED GASTRIC ULCER IN RATS

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ABSTRACT

To evaluate in-vivo antiulcer potential of crude extracts of the indigenous medicinal plant, Cuscuta reflexa, belonging to family Convolvulaceae. The effect of alcoholic and aqueous extracts of Cuscuta reflexa was investigated in rats to evaluate the anti-ulcer activity by using pyloric ligation model experimentally induced gastric ulcer. The parameters taken to assess anti-ulcer activity were volume of gastric acid secretion, ulcer index, free acidity and total acidity. Acute toxicity studies were also performed in mice. The extracts were also analysed Phytochemically. At the end of experimental period, rats were anaesthetized and sacrificed. The stomach was removed and incised to collect the gastric juice for determination of acidity. The stomach from each group was evaluated for ulcer index and percent protection. Phytochemical investigations revealed the presence of carbohydrates, fixed oils, fats, glycosides, saponins, flavonoids, tannins, alkaloids and sterols in alcoholic and aqueous extracts of Cuscuta reflexa. LD\textsubscript{50} studies for both the extracts upto the maximum of 2000 mg/kg dose level no mortality was observed in any of the animals that indicate their practically nontoxic in nature. Oral administration of alcoholic and aqueous extracts of Cuscuta reflexa exhibited dose dependent significant protection in the pylorus ligation induced peptic ulcerated animals.

KEYWORDS: Cuscuta reflexa, Ranitidine, stem extracts, pylorus ligation, anti-ulcer activity.

INTRODUCTION

Peptic ulcer is a multifactorial disease distressing about 8-10\% of the global population.\textsuperscript{[1]} It is the erosion in the mucosa of stomach or duodenum and caused by the disruption of defense
and repair mechanism of gastric mucosa.[2] The ulcer in stomach is known as gastric ulcer and that in duodenum is called duodenal ulcer and together it is termed as peptic ulcer.[3] Currently available drugs used for the treatment of peptic ulcer include antacids, H₂ receptors antagonists, proton pump inhibitors and the drugs affecting the defensive mucosal barrier, but the major drawback of the gastric ulcer therapy is that the currently available drugs for peptic ulcer are associated with severe side effects such as headache, indigestion, sedation, confusion etc.[4] Natural products especially medicinal plants and/or herbs having folkloric and traditional uses have shown important role in the treatment of a number of gastrointestinal disorders.[4] Plant derived saponins,[5] tannins,[6] flavonoids,[6,7] essential oils,[8] gums and mucilages,[9] have received considerable attention in recent decades due to their miscellaneous pharmacological properties including antisecretory and gastroprotective activities.

C. reflexa is a leafless, delicate yellow coloured total stem parasite, belonging to the plant family Convolvulaceae. The tiny white flowers appear in bunches. The fruits are pea shaped and seeds are black in colour.[10] It is found throughout India. The plant is acrid; bitter; astringent to the bowels, aphrodisiac, alternative, tonic and useful in diseases of the eye and of the heart, in biliousness, and in "kapha.

The herb has a bitter sharp taste; used as expectorant, carminative, tonic, anthelmintic, diuretic, blood purifier and lessens inflammation. It is also useful in jaundice, pain in the muscles and joints, headache, paralysis and also in lumbago.

It was reported that decoction prepared with stem is useful in constipation, flatulence, liver complaints and bilious affections.

The plant is purgative, it is used externally against itch and internally to protract fevers. The stems are specially useful in bilious disorders.[10,11,12]

This study was conducted to assess in vivo gastroprotective activity and antiulcer potential of crude extracts of Cuscuta reflexa, belonging to plant family Convolvulace.

From the literature it was found that C. reflexa has also been traditionally indicated for treatment of acid peptic disorders. Hence stem extracts of this plant was select for the study of anti-ulcer activity in pylorus ligation induced peptic ulcerated rats.
MATERIALS AND METHODS

Plant material
Stem of C. reflexa collected in the month of May and dried in shade at room temperature then subjected to size reduction to a fine powder with the help of mixer grinder.

Chemicals
Ranitidine was gift sample from Micro Labs- Bangalore.

Animals
Albino rats (Wistar strain) of either sex weighing between 150-200 g and Albino mice 16-25g were used for the study. The animals were acclimatized for 7 days under standard husbandry condition. i.e.

- Room temperature: 26 ± 2°C
- Relative humidity: 45-55%
- Light/ dark cycle: 12:12 h

The animals were fed with a synthetic standard diet from Amrut Laboratories & Pranav Agro Industries Ltd. Sangli. Water was allowed ad libitum under strict hygienic conditions. All animal studies were performed in accordance to guidelines No. 425 of CPCSEA and Institutional Animal Ethical Committee (IAEC) of Sanjay College of Pharmacy, Mathura, Uttar Pradesh (India). CPCSEA registration number was 1334/01/10/CPCSEA and all the procedures were followed as per rules and regulations.

Preparation of extracts

Preparation of alcoholic extract
The stem powder was packed in a soxhlet apparatus and extracted with 95% alcohol for 18 h. Appearance of colourless solvent in the siphon tube was taken as the termination of extraction. The extract was then transferred into the previously weighed empty beaker and evaporated to a thick paste on the water bath, maintained at 50°C to get alcoholic extract. The extract was finally air dried thoroughly to remove all traces of the solvent and the percentage yield was calculated.\[13\]

Preparation of aqueous extract
About 100 g of powder was taken in a round bottom flask (2000 ml) and macerated with 500 ml of distilled water with 10 ml of chloroform (preservative) for 7 days with occasional
shaking for every hour in a closed vessel. Then the marc was removed by filtering the extract and then it was concentrated on a water bath maintained at 50°C.[13]

These two extracts were stored in an airtight containers in a refrigerator below 10°C. The two extracts were examined for their colour and consistency. Their percentage yield was calculated with reference to air-dried powder sample used for the extraction.

Toxicity studies
The acute toxicity of C. reflexa was determined by using albino mice of either sex (16-20 g), maintained under standard husbandry conditions. The animals were fasted for 3 h prior to the experiment and were administered with single dose of individual extracts of C. reflexa and observed for the mortality upto 48 h study period (Short term toxicity). Based on the short-term toxicity profile, the next dose of the individual extracts was determined as per OECD guidelines No. 425. From the LD₅₀ doses 1/20, 1/10 and 1/5 doses were selected and considered as low, medium and high dose respectively.[14]

Pylorus- ligation induced gastric ulcer[15]
Male albino rats weighing 150-200g were selected for pyloric ligation ulcer model. Rats were divided into 08 groups, each group consisting of six animals. Animals were fasted for 24 h. One group received normal saline 2 ml/kg act as a control, the second group received Ranitidine 30 mg/kg by oral route act as a standard and 3 to 8 group received aqueous and alcoholic extracts of Cuscuta reflexa (100, 200 & 300 mg/kg) by oral route, 30 min prior to pyloric ligation. All the Animals were sacrificed 4 h later and the stomach was opened to collect the gastric contents. The total volume of gastric content was measured. The gastric contents were centrifuged at 1000 rpm for 10 min. One ml of the supernatent liquid was pipetted out and diluted to 10 ml with distilled water. The solution was titrated against 0.01N NaOH using Topfer’s reagent as indicator, to the endpoint when the solution turned to orange colour. The volume of NaOH needed was taken as corresponding to the free acidity. Titration was further continued till the solution regained pink colour. The volume of NaOH required was noted and was taken as corresponding to the total acidity. Acidity was expressed as:

\[
\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality} \times 100 \, \text{mEq/l}}{0.1}
\]

Statistical analysis
All the recorded results are expressed as mean ± SEM from 6 animals. Statistical difference in mean was analyzed by using one-way ANOVA (analysis of variance) followed by Post hoc
test (Dunnett’s ‘t’ test). P value >0.05 was considered as non-significant (ns), P<0.05 as significant (*), P<0.01 as more significant (**) and P< 0.001 as highly significant (***)

RESULTS

The preliminary phytochemical analysis of the AESCR and AQESCR revealed the presence of carbohydrates, sterols, flavonoids, glycosides, fixed oils, fats, saponins and alkaloids. The result of oral administration of alcoholic and aqueous extracts at 100, 200 & 400 mg/kg b.w on different parameters in rats represented Table-1.

All the extracts showed the decrease in gastric juice volume on comparision to control group and indicated their anti-secretary effort (Fig.1). Gastric free acidity is increased in control animals due to pylorus ligation. Various extracts of AESRR and AQESCR decreased the gastric free acidity when compared to standard. The alcoholic extract (400mg/kg) showed similar effect that of standard in reducing the gastric free acidity. (Fig.2).

Various extracts of AESRR and AQESCR showed decrease in total acidity as compared to control (Fig.3). The AESRR and AQESCR extract at 400mg/kg reduced the mean ulcer index 53.18% and 64.10% respectively (Fig.4). Percentage curative ratio of AQECR at 400mg/kg was almost comparable to that of standard (Fig.5). AQCR has exhibited better anti-ulcer activity than the AESCR in pylorus ligation induced ulcer model. The control animals had ulcers and haemorrhagic streaks, whereas in animals administered with the extracts of Cuscuta reflexa there was significant reduction in ulcer index. (Fig 6-13)

Table 1- Effect of AESCR and AQESCR against pylorus ligation induced gastric ulcers in rats.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>TREATMENT AND DOSE</th>
<th>GASTRIC VOLUME (ml)</th>
<th>FREE ACIDITY mEq/L</th>
<th>TOTAL ACIDITY mEq/L</th>
<th>ULCER INDEX</th>
<th>% INHIBITION</th>
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<tr>
<td>I</td>
<td>Control (2 ml/kg normal saline p.o)</td>
<td>6.95 ± 0.11</td>
<td>31.33 ± 1.14</td>
<td>82.33 ± 1.89</td>
<td>2.41 ± 0.27</td>
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<tr>
<td>II</td>
<td>Standard (Ranitidine 30mg/kg p.o)</td>
<td>4.28 ± 0.13**</td>
<td>17.33 ± 0.66**</td>
<td>43.50 ± 1.17**</td>
<td>0.50 ± 0.12**</td>
<td>86.67</td>
</tr>
<tr>
<td>III</td>
<td>AESCR (100 mg/kg p.o)</td>
<td>5.91 ± 0.07**</td>
<td>25.00 ± 0.85**</td>
<td>59.66 ± 1.05**</td>
<td>2.16 ± 0.16**</td>
<td>11.02</td>
</tr>
<tr>
<td>IV</td>
<td>AESCR (200 mg/kg p.o)</td>
<td>4.86 ± 0.08**</td>
<td>22.33 ± 0.66**</td>
<td>47.33 ± 2.01**</td>
<td>1.58 ± 0.08**</td>
<td>25.02</td>
</tr>
<tr>
<td>V</td>
<td>AESCR (400 mg/kg p.o)</td>
<td>4.75 ± 0.07**</td>
<td>20.83 ± 1.01**</td>
<td>46.66 ± 0.50**</td>
<td>0.83 ± 0.1**</td>
<td>53.18</td>
</tr>
</tbody>
</table>
AESC- Alcoholic extract of stem of *Cuscuta reflexa*

AQESCR- Aqueous extract of stem of *Cuscuta reflexa*

Results are mean ± S.E.M. (n = 6). Statistical comparison was performed by using ANOVA coupled with student’s t-test. * P<0.05, ** P<0.01, *** P<0.001 were considered statistically significant when compared to control group.

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<td></td>
<td>mg/kg p.o)</td>
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<tr>
<td>VI</td>
<td>AQESCR (100 mg/kg p.o)</td>
<td>5.76 ± 0.12**</td>
<td>23.83 ± 1.35**</td>
<td>58.00 ± 1.39**</td>
<td>2.08 ± 0.2**</td>
</tr>
<tr>
<td>VII</td>
<td>AQESCR (200 mg/kg p.o)</td>
<td>4.75 ± 0.13*</td>
<td>21.83 ± 0.65**</td>
<td>46.66 ± 1.99**</td>
<td>1.41 ± 0.08**</td>
</tr>
<tr>
<td>VIII</td>
<td>AQESCR (400 mg/kg p.o)</td>
<td>4.65 ± 0.10**</td>
<td>20.0 ± 0.73**</td>
<td>46.00 ± 0.96**</td>
<td>0.75 ± 0.11**</td>
</tr>
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**Figure 1.**

**Figure 2.**
Figure 3.

EFFECT OF AESCR AND AQSQR ON TOTAL ACIDITY

Figure 4.

EFFECT OF AESCR AND AQSQR ON ULCER SCORE

Figure 5.

EFFECT OF AESCR AND AQSQR ON % INHIBITION OF ULCERS
DISCUSSION

Gastric acid is an important factor for the genesis of ulceration in pylorus ligation induced ulcers in rats. In this method the accumulation of gastric juice in the stomach causes ulceration. Gastric acid secretion is regulated by many factors including anxiolytic effect on the CNS, vagal activity, cholinergic, histaminergic and gastrinergic neurotransmissions, the activities of various post-synaptic receptors and the proton pump.\cite{16}

It has been proposed that in pyloric ligation, the digestive effect of accumulated gastric juice and interference of gastric blood circulation are responsible for induction of ulceration. The antiulcer activity of \textit{Cuscuta reflexa} extracts in pylorus ligation model is evident from its significant reduction in gastric volume, total acidity, free acidity, ulcer score and increase in pH of gastric juice. Because of animals treated with \textit{Cuscuta reflexa} extracts significantly inhibited the formation of pylorus ulcer in the stomach and also decreased both acid concentration, gastric volume and increased the pH values. It is suggested that \textit{Cuscuta reflexa} extracts can suppress gastric damage induced by aggressive factors.

The preliminary phytochemical studies revealed the presence of flavonoids in extracts of \textit{Cuscuta reflexa} ; various flavonoids have been reported for its anti-ulcerogenic activity with good level of gastric protection.\cite{17,18} So the possible mechanism of antiulcer action of
Cuscuta reflexa may be due to its flavonoid content. In this study we observed that Cuscuta reflexa provides significant anti-ulcer activity against gastric ulcers in rats.

CONCLUSION
The anti gastric ulcer activity of Cuscuta reflexa stem extracts in pylorus ligation model is evident from its significant reduction in gastric volume, free acidity, total acidity, ulcer score and increase in pH when compared to that of standard drug. The anti-gastric ulcer activity of aqueous extract at 400mg/kg is more significant than that of alcoholic extract at 400mg/kg. Thus it has been scientifically proven that these extracts possess energy potential as an antiulcerogenic agent. Hence, the study justifies the traditional uses of the plant against various gastrointestinal disorders. However, further studies are required to be carried out to isolate the purified compound(s) and to explore the possible mechanism(s) responsible for the gastroprotective potential of the plant.

ACKNOWLEDGEMENTS
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CONFLICT OF INTEREST
There is no conflict of interest.

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