Pharmacological evaluation of anti-ulcer activity of *Cymbopogon flexuosus*

Sreedevi Adikay*, T. Santhoshini

**INTRODUCTION**

Peptic ulcer is the most common gastrointestinal disorder in clinical practice. It mostly occurs due to an imbalance between aggressive factor and the maintenance of mucosal integrity through endogenous defense mechanism. The modern medicine has its own limitations especially against ulcers with complex pathology indicating need of substitute medication from the alternative system of medicine. Further folklore of Gautamela, Central America people use *C. flexuosus* to treat ulcer.12 Previous reports suggested that the leaves of *C. flexuosus* exhibited analgesic, anti-inflammatory, antimicrobial, fungicide, febrifuge, anti-oxidant, deodorant, and anticonvulsant activities.34 There were no experimental data to justify the use of the leaves of *C. flexuosus* for its anti-ulcer activity. Hence, the present study was designed for the systematic evaluation of the anti-ulcer activity of ethanol extract of the leaves of *Cymbopogon flexuosus*.

**ABSTRACT**

**Background:** Peptic ulcer disease is a worldwide problem. Currently, there is no cost-effective treatment to relieve pain, heal the ulcer and prevent ulcer recurrence. Hence, there is a dire need to search and find a suitable treatment from natural sources. The present study was designed to investigate the anti-ulcer activity of ethanol extract of the leaves of *Cymbopogon flexuosus*.

**Methods:** The ethanol extract of the leaves of *Cymbopogon flexuosus* was prepared by hot extraction method. Anti-ulcer activity was evaluated in rats and method employed was pylorus ligation. Animals were divided into four groups of six animals each. The animals of Group I served as normal control (vehicle) which received normal saline (5 ml/kg b.wt., p.o). Group II and III received 200 and 400 mg/kg b.wt. of ethanol extract, respectively. The animals of Group IV served as standard control which received ranitidine (15 mg/kg b.d.wt.). At the end of study parameters like ulcer index, free acidity, total acidity, acid volume, and pH were determined.

**Results:** The ethanol extract showed a significant reduction in the total acidity, free acidity, and acid volume. The efficacy of plant extract at high dose was comparable with the standard drug - ranitidine.

**Conclusion:** Our study results support the ethnomedical use of leaves of *C. flexuosus*.

**Keywords:** Anti-ulcer activity, Ranitidine, *Cymbopogon flexuosus*
METHODS

Collection of plant material

The fresh leaves of the plant *C. flexuosus* were purchased from CIMAP, Boduppal, Uppal (PO), Hyderabad. It was identified and authenticated by Dr. Vatsavaya S. Raju, Department of Botany, Kakatiya University, Warangal. All the chemicals used were of analytical grade and purchased from S. D. Fine/Merck (India).

Preparation of extract

The fresh leaves of *C. flexuosus* were shade-dried and grounded in Wiley mill. The powder was defatted with petroleum ether (60-80°C). The defatted marc was air-dried and macerated with ethanol for 12 hrs. Macerated material was allowed for triple solvent extraction with ethanol for 3 hrs. Extract was concentrated under reduced pressure and obtained semisolid mass was used for the study.

Preliminary phytochemical studies

Preliminary phytochemical screening of the *C. flexuosus* was performed for the presence of carbohydrates, alkaloids, terpenoids, glycosides, steroids, flavonoids, and amino acids.

Pharmacological studies

Animals

Healthy adult male albino rats of Wistar strain weighing 150-200 g were used. They were housed in polypropylene cages and maintained under standard conditions (12 hrs light: 12 hrs dark cycle; 25±3°C and 35-60% humidity). Animals had free access to standard lab chow and tap water. The animals were maintained in accordance with CPCSEA guidelines. All the procedures described were reviewed and approved by Institutional Animal Ethical Committee.

Acute toxicity studies

Acute toxicity studies were performed according to the OECD 423 guidelines.

Antioxidant activity

In the present study, anti-ulcer activity was assessed by employing the pylorus ligation model.

Pyloric ligation method

In this method, albino rats were fasted in individual cages for 24 hrs but with free access to water. Just 2 hrs before starting the experiment, water is also removed. Group I which served as negative control received distilled water; animals of Group II received ethanol extract of *C. flexuosus* at the dose of 200 mg/kg and animals of Group III received ethanol extract of *C. flexuosus* at the dose of 400 mg/kg and Group IV served as positive control which received ranitidine (150 mg/kg) 1 hr before pylorus ligation. 1 hr after drug or saline administration, under light ether anesthesia the abdomen was opened by small midline incision below the xiphoid process, pyloric portion of the stomach was slightly lifted out and ligated avoiding traction to the pylorus or damage to its blood supply the stomach is replaced carefully and the abdominal wall closed by interrupted sutures as described by Shay et al. 9

Nineteen hours later, the pylorus ligated rats were sacrificed by ether overdosing, and their stomachs were dissected out after ligating the esophagus at the cardiac end. Each stomach was cut open along the greater curvature, and the contents were collected into a centrifuge tube, then the mucosa was washed under slow running tap water and the number and size of ulceration were scored. 10

The gastric juice collected from each stomach was centrifuged, and its volume was measured. Free and total acidity were estimated titrimetrically with 0.1 N NaOH using methyl orange and phenolphthalein as indicators. 12 The following parameters were also measured:

Ulcer index

Ulcer index was calculated using following formula:

\[ UI = UN + US + UP \times 10^{-1} \]

Where,

\[ UI = \text{Ulcer index} \]

\[ UN = \text{Average number of ulcers per animal} \]

\[ US = \text{Average of severity score} \]

\[ UP = \text{Percentage of animals with ulcer} \]

pH

The contents were drained into a graduated centrifuge tube. The tubes were centrifuged at 3000 rpm for 10 mins, and the centrifuged samples were decanted and analyzed for pH (using a broad range pH paper).

Statistical analysis

The statistical data was presented as mean±standard error mean. Comparison between the treatment groups and control was performed by one-way analysis of variance followed by Bartlett’s test for equal variances.
RESULTS

Preliminary phytochemical studies

Upon preliminary phytochemical analysis, ethanol extract of leaves of *C. flexuosus* gave positive test for the presence of alkaloids, amino acids, flavonoids, steroids, and carbohydrates.

Pharmacological studies

Acute toxicity studies: The ethanol extract was found to be safe since no animal died even at the maximum dose of 2000 mg/kg b.wt.

Tables 1 and 2 represent the anti-ulcer activity of ethanol extract of *C. flexuosus*.

Effect on acid volume

After pylorus ligation, a significant increase in the acid volume was observed in the Group I animals. Group II and III animals which received 200 mg/kg bd.wt. and 400 mg/kg bd.wt. of ethanol extract of *C. flexuosus* exhibited reduction in acid volume when compared with Group I animals.

Effect on pH

Control group animals (Group I) exhibited decreased pH level after pylorus ligation. On oral administration of 200 mg/kg bd.wt. and 400 mg/kg bd.wt. of ethanol extract of *C. flexuosus*, Groups II and III animals showed a significant increase in pH when compared to Group I animals.

Effect on free acidity

A significant increase in the levels of free acidity was observed in the control Group I. Animals of Group II and III which were treated with ethanol extract of *C. flexuosus* exhibited a significant decrease in free acidity when compared to the control Group I.

Effect on total acidity

The total acidity was significantly increased in control Group I on pylorus ligation. Group II and III animals which were treated with 200 mg/kg and 400 mg/kg of ethanol extract of *C. flexuosus* exhibited a significant decrease in total acidity when compared to the control Group I.

Ulcer index

The severity of gastric ulceration in pylorus ligation method was assessed based on the mean ulcer index. Animals which received the ethanol extract at a dose of 200 mg/kg bd.wt., showed a significant decrease in the mean ulcer number, mean ulcer score, mean ulcer percentage and ulcer index when compared with the control group animals.

Group III animals which received the ethanol extract at 400 mg/kg exhibited mean ulcer number of 2.66±0.21, which is almost comparable to that of ranitidine (2.33±0.21) and proved its protective effect against ulcer formation due to pylorus ligation. Mean ulcer score, mean ulcer percentage and ulcer index of Group III animals were decreased when compared to the control group of animals.

DISCUSSION

Peptic ulcer is one of the most frequent diseases of the alimentary tract. Multifactorial elements, including bacterial infection, and genetic-environmental, autoimmune factors, are involved in the development of peptic ulcer. The etiology of peptic ulcer is unknown in most of the cases, yet it is generally accepted that it results from an imbalance between

<table>
<thead>
<tr>
<th>Name of the group</th>
<th>Acid volume</th>
<th>pH</th>
<th>Free acidity</th>
<th>Total acidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>7.83±0.30</td>
<td>1.50±0.22</td>
<td>74.33±1.47</td>
<td>120.30±0.61</td>
</tr>
<tr>
<td>Group II (ethanol extract 200 mg/kg)</td>
<td>4.50±0.22***</td>
<td>3.66±0.21***</td>
<td>49.33±0.21***</td>
<td>62.00±1.00***</td>
</tr>
<tr>
<td>Group III (ethanol extract 400 mg/kg)</td>
<td>2.83±0.30***</td>
<td>4.66±0.21***</td>
<td>41.83±0.60***</td>
<td>56.00±1.41***</td>
</tr>
<tr>
<td>Group IV (ranitidine 50 mg/kg)</td>
<td>1.66±0.21***</td>
<td>6.33±0.21***</td>
<td>37.00±0.63***</td>
<td>41.66±1.05**</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM. ***p<0.001 when compared to control group. SEM: Standard error mean.

<table>
<thead>
<tr>
<th>Name of the group</th>
<th>MUN</th>
<th>MUS</th>
<th>MUP (%)</th>
<th>UI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>10.50±0.61</td>
<td>2.66±0.21</td>
<td>100</td>
<td>11.2</td>
</tr>
<tr>
<td>Group II (ethanol extract 200 mg/kg)</td>
<td>3.66±0.21</td>
<td>0.91±0.20*</td>
<td>66</td>
<td>6.99***</td>
</tr>
<tr>
<td>Group III (ethanol extract 400 mg/kg)</td>
<td>2.66±0.21</td>
<td>0.25±0.11**</td>
<td>50</td>
<td>5.29***</td>
</tr>
<tr>
<td>Group IV (ranitidine 50 mg/kg)</td>
<td>2.33±0.21</td>
<td>0.16±0.10**</td>
<td>33</td>
<td>3.5***</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM, *p<0.05, **p<0.01, ***p<0.001 when compared to control group. SEM: Standard error mean, MUN: Mean ulcer number, MUP: Mean ulcer percentage, MUS: Mean ulcer score, UI: Ulcer index.
aggressive factors and the maintenance of mucosal integrity through the endogenous defense mechanism.\textsuperscript{15,16} To regain the balance, different therapeutic agents are used to inhibit the gastric acid secretion or to boost the mucosal defense mechanism by increasing mucous production, stabilizing the surface epithelial cells or interfering with the prostaglandin synthesis.\textsuperscript{22} But many of these drugs suffer from incidence of relapses, adverse effects, and danger of drug interactions during the ulcer therapy. Thus, complementary and alternative medicine which covers a range of healing therapies such as herbs, nutritional supplements, diet, etc., are gaining importance.

Indian medicinal plants and their derivatives have been an invaluable source of therapeutic agents to treat various disorders including ulcers. Several plant species like \textit{Cassia auriculata}, \textit{Echinops persicus}, \textit{Rubia cordifolia}, \textit{Terminalia chebula}, \textit{Ficus religiosa}, \textit{Scoparia dulcis}\textsuperscript{18-23} have shown encouraging findings. \textit{C. flexuosus} is one such plant, and folklore people of Goutamela used this for the treatment of ulcers.\textsuperscript{12} Hence, the present study was oriented to evaluate the phytochemical studies and anti-ulcer activity of ethanol extract of \textit{C. flexuosus} against pylorus ligated ulceration.

Anti-ulcer activity of ethanol extract of \textit{C. flexuosus} was tested at two dose levels, i.e. 200 mg/kg bd.wt. and 400 mg/kg bd.wt. and ranitidine was used as standard drug.

In the pylorus ligation model, digestive effect of the accumulated gastric juice is believed to be responsible for producing ulcers, in addition gastric acid secretion reflex or neurogenic effect has also been suggested to play an important role in the formation of gastric ulcers.\textsuperscript{24}

Gastric acid secretion is mainly under vagal control and over activity of the vagus also contributes to ulcer formation in the pylorus ligation model.\textsuperscript{21} Vagal stimulation increases acetylcholine that acts directly on the muscarinic receptors on parietal cells and secretes hydrochloric acid through a calcium-dependent pathway.\textsuperscript{26}

In the present study, extract decreased the gastric juice volume which may be because of its anti-secretory mechanism.

Ethanol extract showed elevation in pH indicating its capacity to reduce the acidity of the gastric juice when compared to control animals. The mean ulcer number value of ethanol extract at 400 mg/kg is almost equipotent as that of ranitidine. The extract of \textit{C. flexuosus} has significantly exhibited a significant decrease in both total acidity and also free acidity in pylorus ligation model. The reduction in acid output, peptic activity and increase in mucus secretion were the major mechanisms behind the protection shown in the pylorus ligation model. The reduced acid output measured after pyloric ligation indicates the effect of the extract’s protective mechanism on gastric mucosa, causing an inhibition of gastric secretion. The results of the present study thus exemplify the anti-ulcer potential of \textit{C. flexuosus}.

CONCLUSION

The results of this study demonstrate that the ethanol extract of leaves of \textit{C. flexuosus} has potent anti-ulcer activity on pylorus ligated-induced ulcers in rats. The reduction in acid output, peptic activity and increase in mucus secretion were the major mechanisms behind the protection shown in the pylorus ligation model.

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Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES
