

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

Sreedevi Adikay*, T. Santhoshini

Division of Pharmaceutical Chemistry, Institute of Pharmaceutical Technology, Sri Padmavati Mahila Visvavidyalayam, Tirupati, Andhra Pradesh, India

Received: 02 January 2015

Revised: 21 January 2015

Accepted: 17 February 2015

***Correspondence to:**

Prof. Sreedevi Adikay,
Institute of Pharmaceutical Technology, Sri Padmavati Mahila Visvavidyalayam, Tirupati - 517 502, Andhra Pradesh, India.
Email: sridevitirupati@rediffmail

Copyright: © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

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 bd.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*

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.^{1,2} Previous reports suggested that the leaves of *C. flexuosus* exhibited analgesic, anti-inflammatory, anti-microbial, fungicide, febrifuge, anti-oxidant, deodorant, and anticonvulsant activities.³⁻⁶ 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 *C. 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.⁷

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.⁸

Antiulcer 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 opened 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.¹¹ 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 index¹³

Ulcer index was calculated using following formula:

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

Where,

UI=Ulcer index

UN=Average number of ulcers per animal

US=Average of severity score

UP=Percentage of animals with ulcer

pH¹⁴

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 1: Effect of ethanol extract of *Cymbopogon flexuosus* on acid volume, pH free acidity and total acidity.

Name of the group	Acid volume	pH	Free acidity	Total acidity
Group I	7.83 ± 0.30	1.50 ± 0.22	74.33 ± 1.47	120.30 ± 0.61
Group II (ethanol extract 200 mg/kg)	$4.50 \pm 0.22^{***}$	$3.66 \pm 0.21^{***}$	$49.33 \pm 0.21^{***}$	$62.00 \pm 1.00^{***}$
Group III (ethanol extract 400 mg/kg)	$2.83 \pm 0.30^{***}$	$4.66 \pm 0.21^{***}$	$41.83 \pm 0.60^{***}$	$56.00 \pm 1.41^{***}$
Group IV (ranitidine 50 mg/kg)	$1.66 \pm 0.21^{***}$	$6.33 \pm 0.21^{***}$	$37.00 \pm 0.63^{***}$	$41.66 \pm 1.05^{**}$

Values are expressed as mean \pm SEM. ***($p < 0.001$) when compared to control group. SEM: Standard error mean

Table 2: Effect of *Cymbopogon flexuosus* on ulcer score in pylorus ligation model.

Name of the group	MUN	MUS	MUP (%)	UI
Group I	10.50 ± 0.61	2.66 ± 0.21	100	11.2
Group II (ethanol extract 200 mg/kg)	3.66 ± 0.21	$0.91 \pm 0.20^*$	66	6.99^{***}
Group III (ethanol extract 400 mg/kg)	2.66 ± 0.21	$0.25 \pm 0.11^{**}$	50	5.29^{***}
Group IV (ranitidine 50 mg/kg)	2.33 ± 0.21	$0.16 \pm 0.10^{**}$	33	3.5^{***}

Values are expressed as mean \pm 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.^{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 mucus production, stabilizing the surface epithelial cells or interfering with the prostaglandin synthesis.¹⁷ 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 *Cassia auriculata*, *Echinops persicus*, *Rubia cordifolia*, *Termanilia chebula*, *Ficus religiosa*, *Scoparia dulcis*¹⁸⁻²³ have shown encouraging findings. *C. flexuosus* is one such plant, and folklore people of Goutamela used this for the treatment of ulcers.^{1,2} Hence, the present study was oriented to evaluate the phytochemical studies and anti-ulcer activity of ethanol extract of *C. flexuosus* against pylorus ligated ulceration.

Anti-ulcer activity of ethanol extract of *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.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.²⁵ Vagal stimulation increases acetylcholine that acts directly on the muscarinic receptors on parietal cells and secretes hydrochloric acid through a calcium-dependent pathway.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 *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 *C. flexuosus*.

CONCLUSION

The results of this study demonstrate that the ethanol extract of leaves of *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.

Funding: No funding sources

Conflict of interest: None declared

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

REFERENCES

1. Devi RC, Sim SM, Ismail R. Spasmolytic effect of citral and extracts of *Cymbopogon citratus* on isolated rabbit ileum. *J Smooth Muscle Res.* 2011;47(5):143-56.
2. Borrelli F, Izzo AA. The plant kingdom as a source of anti-ulcer remedies. *Phytother Res.* 2000;14(8):581-91.
3. Chandrashekhar KS, Prasanna KS. Analgesic and anti-inflammatory activities of the essential oil from *Cymbopogon flexuosus*. *Pharmacogn J.* 2010;2(14):23-5.
4. Silva Cde B, Guterres SS, Weisheimer V, Schapoval EE. Antifungal activity of the lemongrass oil and citral against *Candida* spp. *Braz J Infect Dis.* 2008;12(1):63-6.
5. Adinarayana G, Rahul G, Ravi Kiran S, Syamsundar KV, Rao RBR. Evaluation of antimicrobial potential of field distilled and water soluble essential oils of *Cymbopogon flexuosus*. *J Pharmacogn.* 2012;3(2):142-6.
6. Quintans-Junior LJ, Guimaraes AG, Araujo BES, Oliveira GF, Santana MT, Moreira FV, et al. Carvacrol, (-)-borneol and citral reduce convulsant activity in rodents. *Afr J Biotechnol.* 2010;9(39):6566-72.
7. Harborne JB, editors. *Phytochemical Methods: a Guide to Modern Techniques of Plant Analysis*. 3rd Edition. London: Chapman and Hall; 1998: 302.
8. OECD Test Guideline 423. *OECD Guideline for Testing of Chemicals. Acute Oral Toxicity- Acute Toxic Class*, 2001. Available at http://www.oecd.org/document/40/0,3343,en_2649_34377_37051368_1_1_1_1,00.html. Accessed 02 Nov 2014.
9. Shay H, Komarow SA, Fels SS, Meranze D, Gruenstein M, Siplet H. A simple method for the uniform production of gastric ulceration in the rat. *Gastroenterology.* 1945;5:43-61.
10. Ahamthulla M, Asad M, Satya Prasad V. Antiulcer activity of *Allium sativum* bulb juice in rats. *Saudi Pharm J.* 2009;17(1):70-7.
11. Susan G, Sathimoorthy A, Sathimoorthy SS. Effect of alpha tocopherol on gastric ulcers induced by pylori ligation in rats. *Indian J Pharmacol.* 1990;31:431-3.
12. Gregory M, Vithalrao KP, Franklin G, Kalaichelavan V. Anti-ulcer (ulcer-preventive)activity of *Ficus arnottiana Miq.* (Moraceae) leaf methanolic extract. *Am J Pharmacol Toxicol.* 2009;4(3):89-93.
13. Rao ChV, Ojha SK, Radhakrishnan K, Govindarajan R, Rastogi S, Mehrotra S, et al. Antiulcer activity of

- Utricularia salicifolia* rhizome extract. J Ethnopharmacol. 2004;91(2-3):243-9.
- 14. Parmar NS, Desai JK. A review of the current methodology for the evaluation of gastric and duodenal anti-ulcer agents. Indian J Pharmacol. 1993;25:120-35.
 - 15. Piper DW, Stiel DD. Pathogenesis of chronic peptic ulcer, current thinking and clinical implications. Med Prog. 1986;2:7-10.
 - 16. de Andrade SF, Lemos M, Comunello E, Noldin VF, Filho VC, Niero R. Evaluation of the antiulcerogenic activity of *Maytenus robusta* (Celastraceae) in different experimental ulcer models. J Ethnopharmacol. 2007;113(2):252-7.
 - 17. Ganser AL, Forte JG. K⁺-stimulated ATPase in purified microsomes of bullfrog oxyntic cells. Biochim Biophys Acta. 1973;307(1):169-80.
 - 18. Ahmed MF, Thayyil H, Rasheed AS, Ibrahim M. Anti-ulcer activity of *Cassia auriculata* leaf extract. Pharmacognosy. 2010;2(16):53-57.
 - 19. Rad AA, Najafzadeh-Varzi H, Farajzadeh-Sheikh A. Evaluation of anti-ulcer activity of *Echinops persicus* on experimental gastric ulcer models in rats. Vet Res Forum. 2010;1(3):188-91.
 - 20. Deoda RS, Kumar D, Bhujbal SS. Gastroprotective effect of *Rubia cordifolia* Linn. on aspirin plus pylorus-ligated ulcer. Evid Based Complement Alternat Med 2011;2011:541624.
 - 21. Raju D, Ilango K, Chitra V, Ashish K. Evaluation of anti-ulcer activity of methanolic extract of *Terminalia chebula* fruits in experimental rats. J Pharm Sci Res. 2009;1(3):101-7.
 - 22. Gregory M, Divya B, Mary RA, Hipolith Viji MM, Kalaichelvan VK, Palanivel V. Anti-ulcer activity of *Ficus religiosa* leaf ethanolic extract. Asian Pac J Trop Biomed. 2013;3(7):554-56.
 - 23. Babincová M, Schronerová K, Sourivong P. Antiulcer activity of water extract of *Scoparia dulcis*. Fitoterapia. 2008;79(7-8):587-8.
 - 24. Goswami M, Kulshreshtha M, Rao CV, Yadav S, Yadav S. Anti-ulcer potential of *Lawsonia inermis* L. leaves against gastric ulcers in rats. J Appl Pharm Sci. 2011;1:69-72.
 - 25. Ogle CW, Cho CH, Tong MC, Koo MW. The influence of verapamil on the gastric effects of stress in rats. Eur J Pharmacol. 1985;112(3):399-404.
 - 26. Jain SM, Parmar NS, Santani DD. Gastric anti-ulcer activity of calcium channel blockers in rats. Indian J Pharmacol. 1994;26:29-34.

doi: 10.5455/2319-2003.ijbcp20150402

Cite this article as: Adikay S, Santhoshini T. Pharmacological evaluation of anti-ulcer activity of *Cymbopogon flexuosus*. Int J Basic Clin Pharmacol 2015;4:208-12.