Research Article

Pharmacognostical and phytochemical studies and evaluation of anti-ulcer activity of *Coscinium fenestratum* (gaertn) colebr

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ABSTRACT

Gastric ulcer is one of the most prevalent gastrointestinal disorders, which affects approximately 5-10% of people during their life. In recent years, abundant work has been carried out on herbal medicine to clarify their potential efficacy in gastric ulcer prevention or management. This study gives an account on its phytochemical profile and antiulcer activity of ethanolic extract of *Coscinium fenestratum* (CF) (Gaertn) colebr. (Family: Menispermaceae) stem bark in Aspirin and pylorus ligated induced peptic ulceration in the albino rats. The preliminary phytochemical screenings of various extracts were performed to establish the pharmacognostical profile of the stem-bark. Preliminary ethanol extracts of CF (Gaertn) colebr. were subjected to the acute oral toxicity study according to the OECD Guideline No. 425. Based on which, three dose levels, i.e., 100, 200 and 400 mg/kg were selected for the further study. In ulcer model, various parameters were studied *viz*., gastric volume, pH, total acidity, free acidity, and ulcer index. Ranitidine at 50 mg/kg used as the standard drug. The ethanolic extract of CF at the dose of 100, 200 and 400 mg/kg treated groups offered 1.54%, 9.88% and 51.85% ulcer protection in aspirin + pylorus ligation induced peptic ulcers.

Keywords: Antiulcer activity, *Coscinium fenestratum* (Gaertn) colebr., phytochemical profile

Introduction

A peptic ulcer is the most common gastrointestinal disorder in clinical practice.[1] It is a conglomerate of heterogeneous disorders, which manifests itself as a break in the lining of the gastrointestinal mucosa bathed by acid or pepsin.[2] Gastric and duodenal ulcers are illnesses that affect a considerable number of people in the world, and some authors consider gastric ulcer as the new "plague" of the 21st century.[3] The pathophysiology of gastric ulcer has generally focused on the imbalance between aggressive and protective factors in the stomach such as acid, pepsin secretion, mucosal barrier, mucus secretion, blood flow, cellular regeneration, prostaglandins, and epidermal growth factors. The reactive oxygen species, especially hydroxyl radical, plays a major role in causing oxidative damage of mucosa in all types of ulcers.[4,5]

Herbal drugs obtained from the plant source are relatively less expensive, safe, and possess good tolerability even in higher doses.[6] Plants extracts, however, are some of the attractive sources of new drug and have shown to produce promising results in the treatment of gastric ulcers.[7]

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Materials and Methods

Plant material

The stem barks of the plant *Coscinium fenestratum* (CF) were purchased from the Nagarjuna Herbal Concentrates Ltd. Alakode, Kerala. The plant was identified and authenticated from in Agharkar Research Institute (An Autonomous Institute of Department of Science and Technology, Government of India) G. G. Agharkar Road, Pune - 411004, India.

Extraction

Stem barks were extracted by cold maceration method. The stem barks were dried in shadow. The stem bark was powdered and was passed through no. 85 mesh, weighed and then used for extraction.

Maceration of dried, powdered stem bark of CF was carried out at room temperature for 72 h using 95% ethanol. The resulting macerate was concentrated under reduced pressure using rotary vacuum evaporator to get the syrupy liquid. The syrupy liquid was transferred in Petri dishes and allowed to dry in an oven for about 2-3 h at below 30°C. The amount of extract was weighed and stored in airtight bottle at 5-7°C.

Animals

Swiss albino mice of inbred colony of either sex weighing 18-25 g were obtained from National Toxicology Center, Pune, and were housed in groups of 5-6. Mice were maintained under standard laboratory conditions. All mice were fed pelleted diet, and water *ad-libitum*. Mice were maintained at 22 ± 1°C with 60% relative humidity and kept under 12 h light:dark cycle. The animals were allowed to acclimatize to laboratory conditions before experimentation. All experiments were conducted during the light period of 12-h of the day/night cycle.

Preliminary phytochemical screening

The preliminary phytochemical screening was studied.[8]

Toxicity study

Acute toxicity studies conducted as per the internationally accepted protocol drawn under the OECD guidelines 420 in Swiss albino mice at a dose level of crude extract 2000 mg/kg.

Acute toxicity study[9]

Acute toxicity study for the test drugs was carried out according to OECD guidelines 423. To find the LD_{50} of ethanolic extract of CF, six groups of mice, containing six in each group, were given an ethanolic extract of CF at the doses of 500, 1000, and 2000 mg/kg orally. The animals were observed for 5 min every 30 min till 2 h and then at 4, 8 and 24 h after treatment for any behavioral changes/mortality. They were further observed daily for 7 days for mortality. No mortality up to 7 days after treatment was observed with the ethanolic extract of CF, and therefore, was found safe up to dose of 2000 mg/kg.

Dose selection

Dose was selected based on acute oral toxicity study done on ethanolic extract of CF. Extract was found to be safe up to the dose level 2000 mg/kg. There was no behavioral abnormality and zero mortality was recorded till 48 h post treatment with no signs of acute toxicity. Therefore, $1/10^{th}$ of the dose 2000 mg/kg of ethanolic extract of CF was selected, i.e., 200 mg/kg of ethanolic extract of CF as middle dose in rats. The following regime was followed:

Rats: 100, 200, 400 (mg/kg p.o.).

Routes of administration

Ethanolic extract of CF stem bark was suspended with 1% sodium carboxymethyl cellulose (sodium CMC) in distilled water and given orally to all the animals of respective groups.

Antiulcer activity

Statistical evaluation

Data of independent observations are shown as mean ± standard error of mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) and Student's *t*-test. $P < 0.05$ was considered statistically insignificant.

Data were expressed as mean ± SEM, compared with normal control by unpaired *t*-test ($^{\#}P < 0.05$) and ulcerated control by one-way ANOVA followed by Dunnett's test where Group-III, IV, V, VI compared with Group-II ($^{*}P < 0.05$, $^{**}P < 0.01$).

Results

Preliminary phytochemical screening

Preliminary phytochemical study shows that presence of reducing sugar, non reducing sugar, proteins, volatile oil, glycosides and alkaloids in crude extract which was shown in Table 2.

Antiulcer activity

Aspirin + pylorus ligation induced peptic ulcer

Data of Aspirin+pylorus ligation induced peptic ulcer in rat were shown in Table 1. In aspirin + pylorus ligation induced peptic ulcer method, aspirin has induced ulcerative condition in rats. Ulcerated control group showed significant ($P < 0.05$) increase in ulcer index as compared to normal control group. Normal control group offered 95.45% ulcer protection in aspirin + pylorus ligation induced peptic ulcers (Figure 1).

Ranitidine-treated groups showed significant ($P < 0.01$) decrease in ulcer index as compared to ulcerated control. Ranitidine offered 69.95% protection in aspirin + pylorus ligation induced peptic ulcers.

Animals treated with the ethanolic extract of CF at the dose of 100 mg/kg did not show a significant decrease in ulcer index as compared to ulcerated control.

Animals treated with the ethanolic extract of CF at the dose of 200 mg/kg showed significant ($P < 0.05$) and 400 mg/kg showed significant ($P < 0.01$) decrease in ulcer index as compared to ulcerated control (Table 1).

The ethanolic extract of CF at the dose of 100, 200 and 400 mg/kg treated groups offered 1.54%, 9.88% and 51.85% ulcer protection, respectively, in aspirin + pylorus ligation induced peptic ulcers.

Discussion

Peptic ulcer therapy has undergone many strides over the last few years, and a number of drugs are now available for treatment. Reports on a clinical evaluation of these drugs show that there are incidences of relapses and adverse effects and danger of drug interactions during ulcer therapy. Hence, the search for an ideal antiulcer drug continues

Table 1: Aspirin+pylorus ligation induced peptic ulcers in rat[10]

Groups (*n*=6)	Treatment (1st day-7th day)	Evaluation parameters
Group-I (NC)	Sodium CMC (1% 10 ml/kg p. o.)	Biochemical parameters such as: Ulcer index, Gastric wall mucus, pH of gastric juice, Total acidity, Volume of gastric juice, Peptic activity, In vivo Antioxidant activity
Group-II (UC)	Sodium CMC (1%, 10 ml/kg p. o.)+aspirin (200 mg/kg, p.o.)	
Group-III (Standard)	Ranitidine (50 mg/kg, p.o.)+aspirin (200 mg/kg, p.o.)	
Group-IV (CF-100)	Ethanolic extract of CF at 100 mg/kg, p.o.+aspirin (200 mg/kg, p.o.)	
Group-V (CF-200)	Ethanolic extract of CF at 200 mg/kg, p.o.+aspirin (200 mg/kg, p.o.)	
Group-VI (CF-400)	Ethanolic extract of CF at 400 mg/kg, p.o.+aspirin (200 mg/kg, p.o.)	

NC: Normal control, UC: Ulcerated control

Table 2: Preliminary phytochemical evaluation of crude drug

Tests	Crude extract	Non-alkaloidal fraction	Alkaloidal fraction
Tests for carbohydrates	+	+	-
Reducing sugars	+	-	-
Monosaccharides	-	-	-
Pentose sugars	-	-	-
Hexose sugars	-	-	-
Non-reducing sugars	+	+	-
Starch	+	+	-
Gums	-	-	-
Mucilage	+	+	-
Tests for proteins	+	+	-
Test for amino acids	-	-	-
Tyrosine	-	-	-
Tryptophan	-	-	-
Cysteine	-	-	-
Test for fats and oils	+	+	-
Test for steroids	-	-	-
Test for volatile oils	+	+	-
Test for glycosides	+	+	-
Cardiac glycosides	-	-	-
Anthraquinones	-	-	-
Saponin glycosides	+	+	-
Cynogenetic glycosides	-	-	-
Coumarin glycosides	-	-	-
Flavonoids	-	-	-
Test for alkaloids	+	-	+
Test for tanins	-	-	-
Test for organic acids			
Oxalic acid	-	-	-
Tartaric acid	-	-	-
Citric acid	-	-	-
Malic acid	-	-	-
Test for vitamins			
Ascorbic acid/vitamin C	-	-	-

and has also been extended to herbal drugs in search for new and novel molecules, which afford better protection and decrease the incidence of relapse.[10]

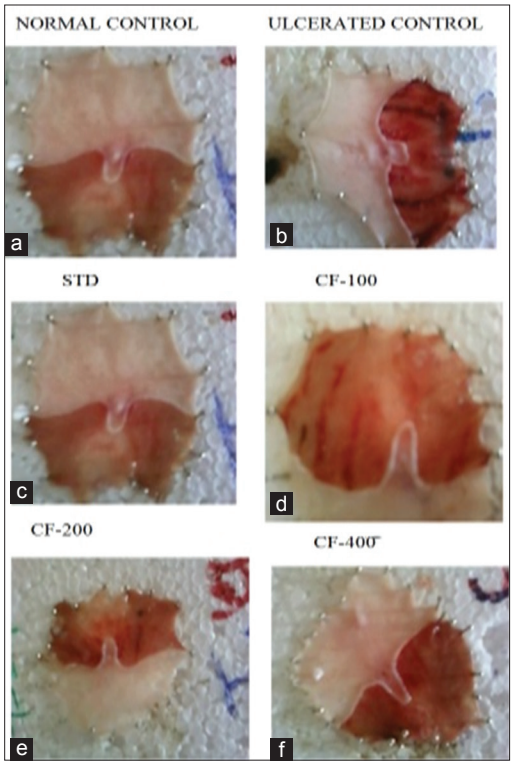


Figure 1: Evaluation of ulcer index of stomach of rats in aspirin+pylorus ligation induced peptic ulcer. (a) Normal control, (b) ulcerated control, (c) standard, (d) CF-100, (e) CF-200, (f) CF-400

Traditionally, CF is being used in the treatment of ulcer.[6] Thus in the present investigation, CF was evaluated for experimentally induced peptic ulcer.

Acute toxicity studies aim at establishing the therapeutic index, i.e., the ratio between the pharmacologically effective dose and the lethal dose and also to perform the primary screening. The ethanolic extracts of the plant CF were found to be safe up to 2000 mg/kg (Table 3).

Aspirin + pylorus ligation induced peptic ulcers

A nonsteroidal anti-inflammatory drug (NSAID) as like aspirin, induced gastroduodenal ulceration via its ability to suppress prostaglandin synthesis. The suppression of prostaglandin, which plays a key role in stimulating the secretion of bicarbonate and mucus,

maintaining mucosal blood flow and regulating mucosal cell turnover and repair, results in increase susceptibility to mucosal injury and gastroduodenal ulceration.[11] In pyloric ligation, the digestive effect of accumulated gastric juice and interference of gastric blood circulation are responsible for induction of ulceration.[12]

Table 3: Effect of the ethanolic extract of *Coscinium fenestratum* on ulcer index and ulcer percentage protection in aspirin+pylorus ligation induced peptic ulcers

Groups (*n*=6)	Ulcer index	Ulcer percentage protection (%)
Group-I (NC)	2.65±0.76	95.45
Group-II (UC)	58.25±1.17$^{\#}$	-
Group-III (Standard)	17.50±0.99**	69.95
Group-IV (CF-100)	57.35±1.61	1.54
Group-V (CF-200)	52.49±1.90*	9.88
Group-VI (CF-400)	28.05±1.77**	51.85

Data was expressed as mean±SEM, where, $^{\#}P$<0.001 when compared with normal control; *P<0.05 and **P<0.01 when compared with ulcerated control. One-way ANOVA followed by Dunnett's test.
NC: Normal control, UC: Ulcerated control

Table 4: Effect of the ethanolic extract of *Coscinium fenestratum* on gastric volume, pH and total acidity in aspirin+pylorus ligation induced peptic ulcers

Groups (*n*=6)	Gastric volume (ml/100 g)	pH	Total acidity (meq/L/4 h)
Group-I (NC)	2.98±0.38	4.98±0.13	987.54±112.8
Group-II (UC)	6±0.44$^{\#}$	1.53±0.09$^{\#}$	2420±102.51$^{\#}$
Group-III (Standard)	2.49±0.40**	5.3±0.22**	800±30.26**
Group-IV (CF-100)	5.6±0.36	2.185±0.18*	2040±107.59
Group-V (CF-200	4.16±0.30*	3.6±0.18**	1233.33±116.05**
Group-VI (CF-400)	3±0.22**	4.66±0.23**	1033.66±88.78**

Data was expressed as mean±SEM, where, $^{\#}P$<0.001 when compared with normal control; *P<0.05 and **P<0.01 when compared with ulcerated control. One-way ANOVA followed by Dunnett's test.
NC: Normal control, UC: Ulcerated control

Table 5: Effect of the ethanolic extract of *Coscinium fenestratum* on gastric wall mucus and peptic activity in aspirin+pylorus ligation induced peptic ulcers

Groups (*n*=6)	Gastric wall mucus (µg alcian blue/g tissue)	Peptic activity (µmoles tyrosine/ml)
Group-I (NC)	81.22±1.98	9.98±1.32
Group-II (UC)	70.05±2.44$^{\#}$	21.33±1.63$^{\#}$
Group-III (Standard)	83.12±1.70**	7.175±1.115**
Group-IV (CF-100)	71.24±2.58	19.02±1.45
Group-V (CF-200)	77.63±1.65*	15.25±1.16*
Group-VI (CF-400)	80.22±1.52**	10.40±1.5627**

Data was expressed as mean±SEM, where, $^{\#}P$<0.001 when compared with normal control; *P<0.05 and **P<0.01 when compared with ulcerated control. One-way ANOVA followed by Dunnett's test.
NC: Normal control, UC: Ulcerated control

Effect on gastric volume, pH, total acidity, ulcer index, and ulcer percentage protection

The etiology behind aspirin + pylorus ligation induced ulcer includes an increase in the acid secretion, which in turn cause increase in gastric volume, low pH, and increase in free and total acidity resulting into increase in ulcer index. The ethanolic extract of CF significantly increased the pH of gastric juice and decreased the gastric volume (Table 4) and total acidity when compared with ulcerated control group. The pH of the ethanolic extract of CF was found to be weak basic. Thus contributing in the acid neutralization may be responsible for the gastroprotective effect by the ethanolic extract of CF in peptic ulcer (Graph 2).

Effect on gastric wall mucus

The results of effect of ethanol extract on Coscinium fenestratum on gastric volume, pH and total acidity in aspirin+pylorus ligation induced peptic ulcer were shown in Table 5.

Aspirin is known to induce gastric damage by suppression of prostaglandins. Thus, NSAIDs topically act on tissue to disrupt the hydrophobic protective lining of the mucus gel layer.[13] In this study, prior administration of the ethanolic extract of CF to the group of rats treated with aspirin showed significant increase gastric wall mucus in comparison to ulcerated control group. This may be due to decrease in leakage of protein content into the gastric juice which further strengthening the mucosal barrier and increase in its resistance to the damaging effect of aspirin (Graph 3). This gastroprotective effect of the ethanolic extract of CF by increase gastric wall mucus may further contribute its use in peptic ulcer.

Effect on peptic activity

Pepsin is an enzyme whose zymogen (pepsinogen) is released by the chief cells in the stomach and that degrades food proteins into peptides. Pepsinogen is activated by hydrochloric acid (HCl) into pepsin, which is released from parietal cells in the stomach lining. It was observed that the ethanolic extract of CF pretreatment has reversed the increased peptic activity by decreasing gastric acid and pepsin secretion associated with aspirin which may further contribute in the treatment of peptic ulcers.

Table 6: Effect of the ethanolic extract of *Coscinium fenestratum* on antioxidant enzyme levels in stomach in aspirin+pylorus ligation induced peptic ulcers

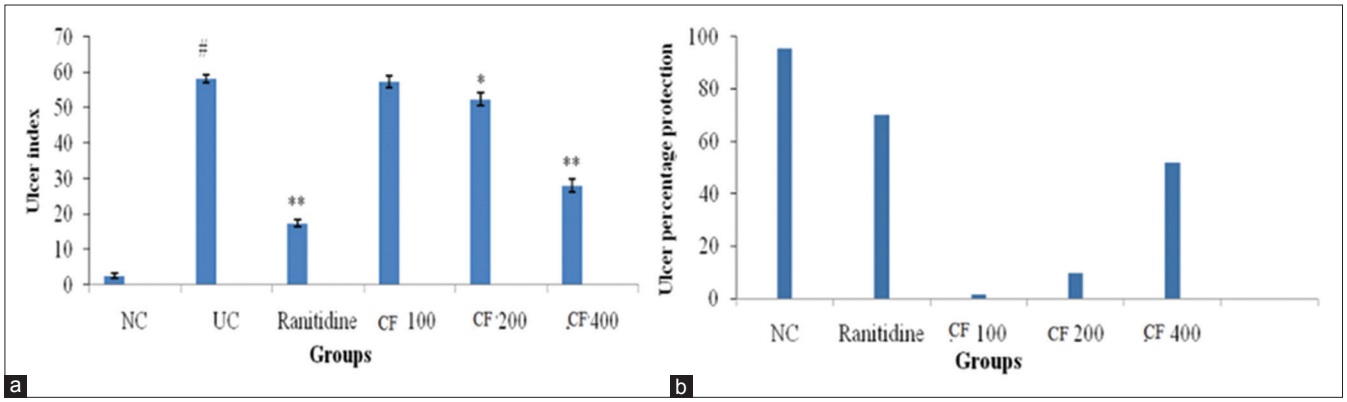
Groups (*n*=6)	LPO (nmoles MDA/g tissue)	SOD (Units/g tissue)	GSH (µmoles/g tissue)	Catalase (µmoles H_2O_2 consumed/g tissue)
Group-I (NC)	6.72±1.22	9.05±0.88	5.76±1.21	11.27±0.90
Group-II (UC)	17.78±1.44$^{\#\#}$	5.29±0.15$^{\#\#}$	1.82±1.11$^{\#\#}$	7.58±0.52$^{\#\#}$
Group-III (standard)	7.78±2.2*	8.56±0.72*	5.22±1.04*	10.24±0.29**
Group-IV (CF-100)	17.01±2.18	6.78±0.70	1.94±0.60	8.68±0.66
Group-V (CF-200)	15.33±1.08	6.39±0.61	1.99±0.55	8.90±0.61
Group-VI (CF-400)	9.18±2.42*	7.21±0.42*	4.28±0.88*	9.22±0.23

Data was expressed as mean±SEM, compared with normal control by unpaired *t*-test ($^{\#\#}P$<0.001) and ulcerated control by one-way ANOVA followed by Dunnett's test where Group-III, IV, V, VI compared with Group-II (*P<0.05, **P<0.01). LPO: Lipid peroxidation, SOD: Superoxide dismutase, GSH: Glutathione, SEM: Standard error of mean, NC: Normal control, UC: Ulcerated control

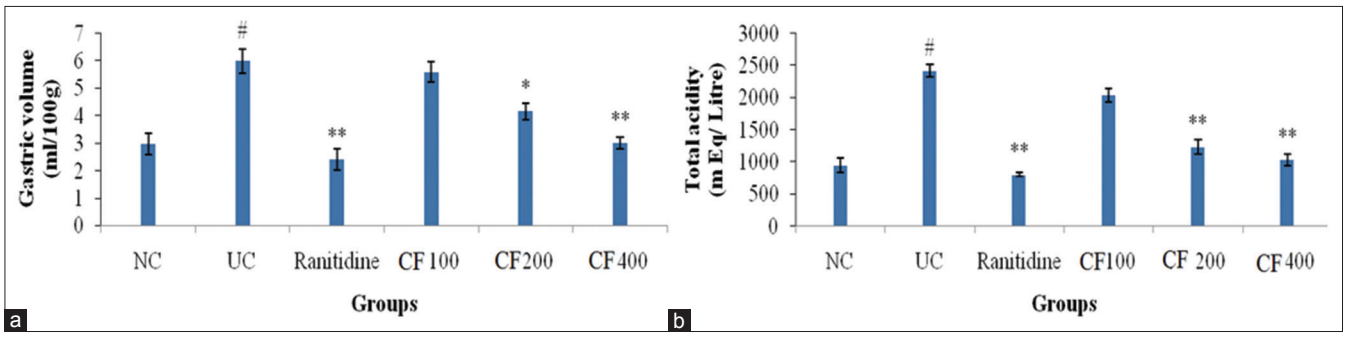
Table 7: Effect of the ethanolic extract of *Coscinium fenestratum* on antioxidant enzyme levels in liver in aspirin+pylorus ligation induced peptic ulcers

Groups (n=6)	LPO (nmoles MDA/g tissue)	SOD (units/g tissue)	GSH (µmoles/g tissue)	Catalase (µmoles H_2O_2 consumed/g tissue)
Group-I (NC)	16.33±1.08	43.78±4.66	13.5±0.81	98.24±2.22
Group-II (UC)	50.00±1.24$^{\#\#}$	9.28±0.55$^{\#\#}$	5.66±0.88$^{\#\#}$	40.78±3.33$^{\#\#}$
Group-III (standard)	25.55±1.92**	40.05±3.43**	12.83±2.01**	91.34±3.01**
Group-IV (CF-100)	48.01±0.96	9.51±0.66	7.83±0.77	42.93±2.52
Group-V (CF-200)	40.50±1.62**	22.54±1.48*	9.06±1.24	56.94±1.44**
Group-VI (CF-400)	35.19±1.25**	28.86±1.99*	12.00±1.33**	70.56±4.23**

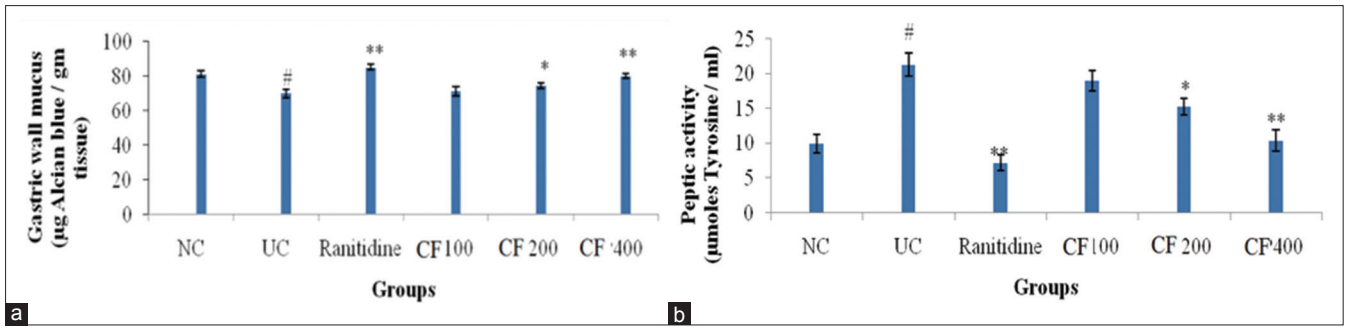
Data was expressed as mean±SEM, compared with normal control by unpaired *t*-test ($^{\#\#}P<0.001$) and ulcerated control by one-way ANOVA followed by Dunnett's test where Group III, IV, V, VI compared with Group II (*$P<0.05$, **$P<0.01$). LPO: Lipid peroxidation, SOD: Superoxide dismutase, GSH: Glutathione, SEM: Standard error of mean, NC: Normal control, UC: Ulcerated control



Graph 1: (a and b) Effect of the ethanolic extract of *Coscinium fenestratum* on ulcer index and ulcer percentage protection in aspirin+pylorus ligation induced peptic ulcers



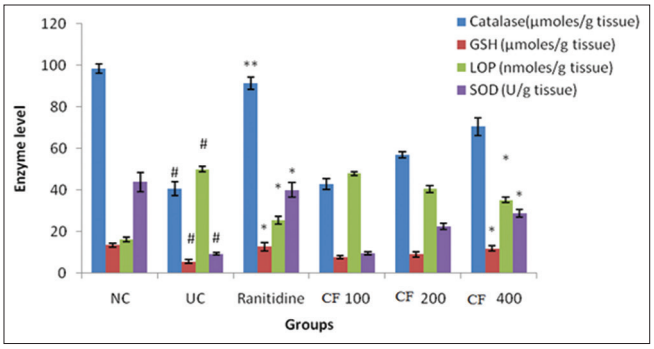
Graph 2: (a and b) Effect of the ethanolic extract of *Coscinium fenestratum* on gastric volume, pH and total acidity in aspirin+pylorus ligation induced peptic ulcers



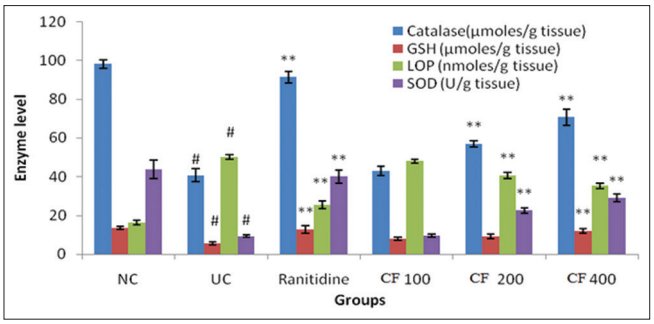
Graph 3: (a and b) Effect of the ethanolic extract of *Coscinium fenestratum* on gastric wall mucus and peptic activity in aspirin+pylorus ligation induced peptic ulcers

Effect antioxidant enzyme levels in stomach and liver

The damage in ulcer involves reactive oxygen species (ROS) apart from acid and pepsin related factors. Increased level of lipid peroxidation (LPO) is due to increase in generation of ROS during stress leading to oxidative damage. Glutathione (GSH) is a major low molecular weight scavenger of free radicals in the cytoplasm and an important inhibitor of free radical mediated LPO which cause ulcer.[4]



Graph 4: Effect of the ethanolic extract of *Coscinium fenestratum* on antioxidant enzyme levels in stomach in aspirin+pylorus ligation induced peptic ulcers



Graph 5: Effect of the ethanolic extract of *Coscinium fenestratum* on antioxidant enzyme levels in liver in aspirin+pylorus ligation induced peptic ulcers

In stomach

In stomach homogenate, the ethanolic extract of CF significantly restored the level of superoxide dismutase (SOD), GSH, and decreased LPO. However, it could not restore the decreased catalase (CAT) level significantly in comparison to ulcerated control.[14]

In liver

Since most of the drugs are metabolized in the liver, the effect of the ethanolic extract of CF on the antioxidant enzyme in the liver was also evaluated. In liver homogenate, CAT, GSH, SOD and LPO levels were restored significantly by the ethanolic extract of CF.

This the antioxidant effect of the ethanolic extract of CF may further contribute in Gastroprotective effect in the treatment of peptic ulcers.

This study thus demonstrates that the plant CF possesses the Antiulcer activity. It may be because of the presence of alkaloids mainly Berberine. It has a wide range of pharmacological and biological activity including anti-inflammatory and antimicrobial properties. In this study, we have shown the beneficial effect of berberine on the ulcer system as ant it is more potent and less toxic.

Conclusion

In this study, the ethanolic extract of leaves of CF was evaluated for peptic ulcer. In aspirin + pylorus ligation induced peptic ulcer, the ethanolic extract of CF has increased the pH of gastric juice, decreased the gastric volume, decreased ulcer index and decreased total acidity by regulating the activity of acid secreting cell (Tables 6 and 7). The ethanolic extract of CF increased gastric wall mucus content and reversed the increased peptic activity. Moreover, the ethanolic extract of CF has significantly restored the level of SOD, GSH, CAT and LPO in stomach and liver (Graphs 4 and 5). Thus, the ethanolic extract of leaves of CF may have antisecretory activity.

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