Research Article

Evaluation of Antiulcer Activity of the Aqueous Extract of *Piper nigrum* and *Ferula foetida*

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ABSTRACT

In this modern era, gastrointestinal disorders are the universal problem. Peptic ulcer is one of the major diseases affecting the human population. It develops due to the imbalance between aggressive factors like acid, pepsin, *H. pylori* and bile salts and defensive factors like mucous, bicarbonate, blood flow, epithelial cell restoration and prostaglandins. The anti-ulcer activity of Aqueous Extract of *Piper nigrum* and *Ferula foetida* (AEPF) was evaluated by using the experimental models of acute gastric lesions induced by aspirin and pylorus ligation in rats, cold restraint stress and cysteamine induced duodenal ulceration. Animals pre-treated with doses of 100 mg/kg, 200 mg/kg and 400 mg/kg of AEPF were statistically analyzed and compared to the standard and control group with the parameters like volume of gastric secretion, total acidity and ulcer index. The results suggested that AEPF significantly decreased volume of gastric acid secretion, total acidity and ulcer index in comparison with standard drug Omeprazole. AEPF shown significant reduction in lesion index, total affected area and percentage of lesion in comparison with control group in aspirin induced ulcer in experimental models. The gastric mucosal protective effect of AEPF is brought by inhibiting the gastric secretion, which shows it may act like a proton pump inhibitor. Thus the present study indicates that AEPF has anti-ulcerogenic potency in aspirin and pylorus ligation, cysteamine and cold restraint stress induced ulcers in rats.

Keywords: Antiulcer, aspirin, cold stress, duodenal ulcer, peptic ulcer, pylorus ligation

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INTRODUCTION

Peptic ulcer is also known as Acid peptic disease (APD), an ulceration of the mucous membrane of the stomach, duodenum or esophagus. An ulcer is a sore or erosion that forms when the lining of the digestive system is corroded by acidic digestive juices and thus extremely painful [1]. It is produced by an imbalance between gastro duodenal mucosal defense mechanisms and the aggressive factors, particularly gastric acid and pepsin [2].

In this modern world gastrointestinal disorders are the universal problem. Nowadays people are subjected to increase in stress due to the modern life style and they often consume fast foods. These factors lead to many kinds of gastro intestinal disorders. About 10% of the population may develop peptic ulcer in their life time [3]. It affects 9.5% among women and 10.5% among men. Duodenal ulcer is most frequent in the individuals of age group 30 to 55 years. In the general population 20-50% is infected with H. pylori, its prevalence increases with age and 15-20% of the infected individuals will develop peptic ulcer [4]. Various antiulcer drugs are available in the market such as H2 receptor antagonist, Proton pump inhibitors, 5-HT4 receptor

agonist, cytoprotectant, healing agents etc. The adverse effects of these drugs are cardiac arrhythmias, blood dyscrasias, hypertension, central nervous system and gastro intestinal disturbances, nephritis, impairment of sexual drive, hepatitis, pancreatitis, increased liver enzyme activity and triglycerides, leucocytopenia and thrombocytopenia, pharyngitis, pruritis and electrolyte imbalance [5]. So, there is a necessity to discover a potent and safe antiulcer drug with less or no side effect.

The *Ferula* genus (*Umbelliferae*) has been found to be a rich source of gum-resin [6]. This resin enjoys a reputation as a folklore medicine [7]. Sedative, carminative, antispasmodic, digestive, expectorant, laxative, analgesic, anthelminitic, antiseptic and diuretic properties have been reported from the *Ferula* genus [8]. Piperine is a nitrogenous pungent substance present in black pepper, obtained from *Piper nigrum* L. (Piperaceae). It has been shown that piperine reduces inflammation and pain processes [9], reported to have anticonvulsant and antiulcer activity [10] and protects the liver. [11] There are no previous reports regarding the anti-ulcer activity, hence in the present study an attempt was made to screen aqueous extract of fruits of *Piper nigrum* and *Ferula foetida* for antiulcer activity using gastric ulceration and duodenal ulceration models.

MATERIALS AND METHODS

Preparation of the plant extract:

The plant material of *Piper nigrum* and *Ferula foetida* is ground to coarse form and this dried powder was used for extraction. Extraction was done by maceration process. The powdered material was taken in the round bottom flask to which sufficient water is added to the round bottom flask as solvent. Equal quantity of crude drug (50g each) in 1000 ml water was taken for maceration process. The whole apparatus kept aside for 3 to 4 days. In-between thoroughly the solvent was mixed for better extraction. After 4 days the contents were filtered and marc was separated. Further concentration of the extract was made by heating and evaporating the solvent kept in a water bath at 40^0C, which finally gave a dark sticky residue. It was stored in an air tight container in a refrigerator.

Animals

Adult male Sprague–Dawley rats (150 ± 10 g body weight) were obtained from the departmental animal facility where they were housed under standard husbandry conditions (25 ± 2 °C temp., 60–70% relative humidity and 12 h photoperiod) with standard rat feed and water *ad libitum*. Experiments were conducted in accordance with the guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India and experimental protocols were approved by the Institutional Animal Ethics Committee (CPCSEA/1217/2008/a).

Experimental method:

Aspirin plus pylorus ligation induced gastric ulcer in rats:

Albino rats were selected and are grouped into seven groups of six animals each.

Group I: Control 1 (No Pylorus ligation) - These rats received 1 ml distilled water.

Group II: Control 2 (With Pylorus ligation) - These rats received 1 ml distilled water.

Group III: Positive Control –Aspirin (200 mg/kg p.o) + Pylorus ligation

Group IV: Standard- Omeprazole (10 mg/kg p.o) + Aspirin (200 mg/kg p.o) + Pylorus ligation

Group V: AEPF 1 (100 mg/kg p.o.) + Aspirin (200 mg/kg p.o) + Pylorus ligation

Group VI: AEPF 2 (200 mg/kg p.o.) + Aspirin (200 mg/kg p.o) + Pylorus ligation

Group VII: AEPF 3 (400 mg/kg p.o.) + Aspirin (200 mg/kg p.o) + Pylorus ligation

Experimental procedure:

In this method albino rats were fasted in individual cages for 24 h. Care was taken to avoid coprophagy. Aspirin was administrated orally in the dose of 200mg/kg in non-fasted rats once daily for 5 days. Test drug and Omeprazole were administrated orally to respective treatment groups 30 min before each Aspirin treatment whereas the control received only distilled water. On the sixth day, pylorus ligation was performed under ether anaesthesia on 36 h fasted rats, immediately after pylorus ligation aspirin treatment was given. Drinking water was withheld after pylorus ligation on the sixth day in each rat and gastric juice was allowed to accumulate for the period of 4 h.

At the end of 4 h after ligation, the animals were sacrificed with excess of anaesthetic ether, and the stomach was dissected out. Gastric juice was collected and its volume was measured. The glandular portion was then exposed and examined for ulceration. Ulcer index was determined.

Cysteamine induced duodenal ulceration:

Albino rats were selected and are grouped into six groups of six animals each.

Group I: Control - These rats received 1 ml distilled water.

Group II: Positive Control Cysteamine (400 mg/kg p.o.)

Group III: Standard-Omeprazole (10 mg/kg p.o.) + Cysteamine (400 mg/kg p.o.)

Group IV: AEPF 1 (100 mg/kg p.o.) + Cysteamine (400 mg/kg p.o.)

Group V: AEPF 2 (200 mg/kg p.o.) + Cysteamine (400 mg/kg p.o.)

Group VI: AEPF 3 (400 mg/kg p.o.) + Cysteamine (400 mg/kg p.o.)

Experimental procedure:

Cysteamine HCl (400 mg/kg, p.o. in 10% aqueous solution) was administered in two doses at an interval of 4 h to produce duodenal ulcers in rats. The extract was given daily for 5 days. Extract or reference drug or control vehicle was administered 30 min before each dose of Cysteamine HCl. All the animals were sacrificed 24 h after the first dose of Cysteamine. Then duodenum was excised carefully and opened along the antimesenteric side. The ulcer score was obtained by measuring the dimensions of the duodenal ulcer(s) in square millimetres and ulcer index was determined.

COLD RESTRAINT STRESS:

Albino rats were selected and are grouped into six groups of six animals each.

Group I: Control - These rats received 1 ml distilled water.

Group II: Positive Control

Group III: Standard-Omeprazole (10 mg/kg p.o)

Group IV: AEPF 1 (100 mg/kg p.o)

Group V: AEPF 2 (200 mg/kg p.o)

Group VI: AEPF 3 (400 mg/kg p.o)

Experimental procedure: Animals were fasted for 24 h prior to the experiment and divided into six groups with six animals in each group. Extract was given daily for 5 days. On the last day after the administration of control vehicle or test drug or reference drug, Stress ulcers were induced after 30 min of extract or Omeprazole treatment; rats were immobilized under light ether anaesthesia and subjected to the cold stress at 4 ± 1°C for 3.5 h. The rats were sacrificed after 3 hrs after the cold restraint stress. Blood was collected by carotid bleeding, allowed to stay at room temperature for some time and then centrifuged at 4000rpm for 15min. Serum was separated from centrifuged blood and stored for estimation of serum biochemical parameters i.e. Glucose, Triglycerides and Cholesterol. The animals were sacrificed under light ether anaesthesia the stomach of each animal was removed and cut along the greater curvature. The extent of gastric damage was assessed. Ulcer index and % Ulcer inhibition were calculated.

Statistical Analysis

The experimental results were expressed as the Mean ± SEM with six rats in each group. The variation between various groups were analyzed statistically using one-way analysis of variance (ANOVA) using the Graph Pad Prism version 5.0, followed by Dunnett's multiple comparison test (DMCT). Results were considered statistically significant when $P < 0.05$.

RESULTS

Effect of aqueous extract of *Piper nigrum* and *Ferula foetida* (AEPF) **on aspirin plus pylorus ligation induced gastric ulceration in rats:**

The aqueous extract of *Piper nigrum* and *Ferula foetida*(AEPF) produced a significant inhibitory action against aspirin plus pylorus ligation induced gastric ulcers. Pretreatment of the aqueous extract of *Piper nigrum* and *Ferula foetida* (AEPF) produced a significant decrease in the ulceration induced by aspirin and pylorus ligation. The standard drug Omeprazole showed significant reduction in ulcer index as compared to control group, whereas AEPF at highest dose also showed significant reduction in ulcer index (**Table 1**).

Table 1: Effect of aqueous extract of *Piper nigrum* and *Ferula foetida*(AEPF) on aspirin plus pylorus ligation induced gastric ulcer in rats

Groups	Treatment	Ulcer index mean±SEM	Total acidity (meq/lit)	Vol of Gastric Juice (ml/100g)
Group 1	No Pylorus ligation (Normal Control)		-	-
Group 2	only Pylorus ligation (Pylorus ligation control)	1.5±0.15*	55.50±3.81 *	1.95±0.076*
Group 3	Aspirin(200mg/kg p.o.) + Pylorus ligation (Positive control)	3.0±0.11	83.166±1.55	2.21±0.11
Group 4	Omeprazole(10mg/kg p.o.)+ Pylorus ligation	1.18±0.06***	49.50±1.17**	1.96±0.20**
Group 5	AEPF 1 (100 mg/kg p.o.) + Aspirin (200 mg/kg p.o.) + Pylorus ligation	1.86±0.15ns	**42.667±6.67ns**	2.217±0.152ns
Group 6	Extract 2 (200 mg/kg p.o.) + Aspirin (200 mg/kg p.o.) + Pylorus ligation	1.65±0.16**	**40.983**±0.643*	1.733±0.170ns
Group 7	Extract3 (400 mg/kg p.o.) + Aspirin (200 mg/kg p.o.) + Pylorus ligation	1.59±0.27***	38.667±0.23 **	1.603±0.128*

Values are expressed as mean ± SEM. Data analysed by one way Annova followed by dunnet's multiple comparision test. ***p<0.001 **p<0.01, *p<0.05 when compare to the Aspirin treated group.

Effect of aqueous extract of *Piper nigrum* and *Ferula foetida* (AEPF) on cysteamine induced duodenal ulceration:

In the cysteamine induced duodenal ulcers oral administration of AEPF at the dose of 100 mg/kg, 200 mg/kg and 400 mg/ kg showed a reduction in ulcer index in a dose dependent manner. AEPF 400 mg/kg produced statistically significant reduction in ulcer score as compared to ulcer control animals. Omeprazole (10 mg/kg) produced significant protection as compared to ulcer control group (**Fig. 1**).

Effect of aqueous extract of *Piper nigrum* and *Ferula foetida*(AEPF) on cold restraint stress induced gastric ulcers:

Pretreatment of the rats with **aqueous extract of *Piper nigrum* and *Ferula foetida*** (AEPF) produced a significant reduction in ulcers when compared to the control group. Even with Omeprazole, significant reduction in ulcers was observed, when compared to the extract (**Table 2, Figure 2**).

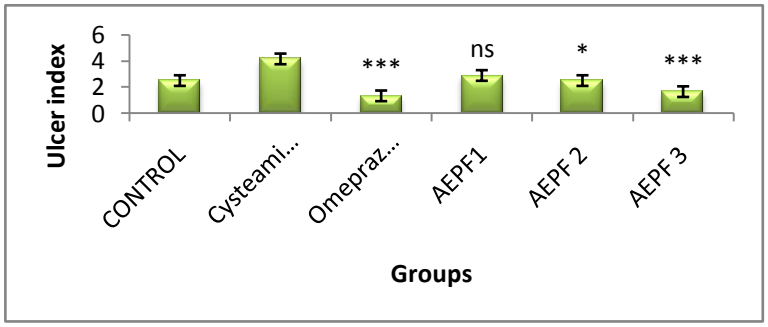

Figure 1: Effect of aqueous extract of *Piper nigrum* and *Ferula foetida*(AEPF) on ulcer index in Cysteamine induced duodenal ulceration.

Values are expressed as mean ± SEM. Data analysed by one way Annova followed by dunnet's multiple comparision test. ***p<0.001, **p<0.01, *p<0.05 and ns –no significance when compared to the Cysteamine treated group.

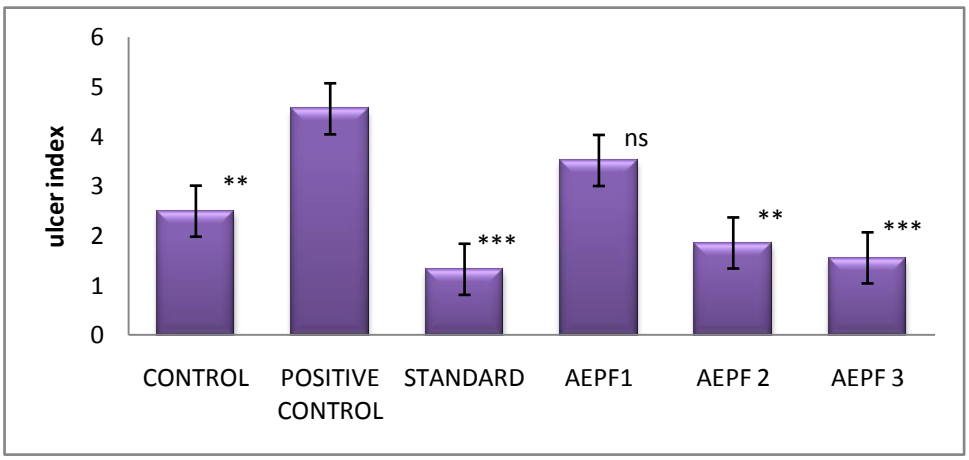

Figure 2: Effect of aqueous extract of *Piper nigrum* and *Ferula foetida* (AEPF) on ulcer index in cold restraint stress induced gastric ulcers.

Values are expressed as mean ± SEM. Data analysed by one way Anova followed by dunnet's multiple comparision test. ***p<0.001, **p<0.01, *p<0.05 and ns –no significance when compared to the cold restraint stress group.

Table 2: Effect of aqueous extract of *Piper nigrum* and *Ferula foetida* (AEPF) on Blood parameters in cold restraint stress induced gastric ulceration model

Groups	Glucose mg/dl	Cholesterol mg/dl	Triglycerides mg/dl
Control	78.03±0.088**	83.18±0.05*	87.49±3.65 *
Cold stress Control	102.9±0.082	99.00±0.02	182.65±1.88
Omeprazole	86.9±0.02***	60.1±0.06**	85.02±3.42**
Extract 100mg/kg p.o	97.4±0.01ns	69.6±0.02ns	143.99±4.01ns
Extract 200mg/kg p.o	92.99±0.2*	66.1±0.05*	95.25±3.79*
Extract 400mg/kg p.o	90.9±0.02***	63.00±0.02**	87.43±1.55**

Values are expressed as mean ± SEM. Data analysed by one way Annova followed by dunnet's multiple comparision test.***p<0.001, **p<0.01, *p<0.05 and ns –no significance when compared to the cold restraint stress group.

DISCUSSION

The results in pyloric ligation model showed significant reduction in basal gastric secretion and inhibition of ulcers by aqueous extract of *Piper nigrum* and *Ferula foetida* (AEPF). This suggests that the antiulcer activity of AEPF on gastric mucosa may be due to the reduction of gastric secretion through one or more of the possible mechanisms [12]. Moreover, gastric acid is an important factor for the genesis of ulceration in pyloric ligation ulcer in rats. Gastric acid secretion is regulated by many factors including anxiety, vagal activity, cholinergic, histaminergic and gastrinergic neurotransmissions, the activities of various post-synaptic receptors and the proton pump. It is therefore, difficult to elucidate the relationship between the mechanisms of inhibition of gastric acid by AEPF.

The antiulcer property of AEPF in pylorus ligation model is evident from its significant reduction in free acidity, total acidity, number of ulcers and ulcer index. AEPF treated animals significantly inhibited the formation of ulcers in the pylorus ligated rats and also decreased both the concentration and increased the P^H, it is suggested that AEPF can suppress gastric damage induced by aggressive factors [13].

The current data clearly demonstrated that AEPF inhibited the aggressive factor, gastric acid secretion. The anti-ulcerogenic effect of the AEPF may be related to its antisecretory action since acid is a major factor in the development of peptic ulcer. The current data also clearly demonstrated that the 400 mg/kg is more effective than the 200 mg/kg and 100mg/kg dose of AEPF and has shown increased p^H and decreased total acidity of gastric fluid.

The ulcerogenic effect of cysteamine is both rapid and constant thus providing a particularly reliable model for investigating the mechanism of duodenal ulcerogenesis and possible means for its prevention. Our results confirmed the suitability of the method, so acute and almost invariably prominent duodenal ulcers were developed in rats that recieved cysteamine HCl 400mg/kg. The exact mechanism of pathogenesis in the cysteamine-induced duodenal ulcer model is not fully known but hypersecretion of gastric acid, deterioration of mucosal resistance and promotion of gastric emptying are among the possible mechanisms [14].

Results also indicated that AEPF extract was effective with doses used in our study. For the main parameters including ulcer area and ulcer index, the effect of larger doses of the extract was comparable with the reference tested drugs. The exact mechanism of action could not be clearly delineated but the candidate plants contains active materials which for most of them ulcer protective properties have been suggested.

A number of interesting biological activities like antiviral, antibacterial, anti-inflammatory and antioxidant are attributed to this compound and a review on its pharmacology indicating a wide therapeutic potential including treating or preventing bronchial asthma, spasmogenic disorders, peptic ulcer, inflammatory disease and atherosclerosis. In addition, antiulcer drugs of plant origin show that triterpenoids, because of their ability to strengthen defensive factors such as stimulation of mucus synthesis or maintenance of the prostaglandins level are potential compounds with antiulcer activity.

The beneficial effects of different single-dose pretreatments with aqueous extract of *Piper nigrum* and *Ferula foetida* (AEPF) in CRS-induced gastric ulcers were recently demonstrated. In this sense, the antiulcer properties AEPF of were investigated *in vivo*, at the level of gastric mucosa. Here, experimental animals were supplemented with AEPF for 5 days.

In the present study, the ulcer protective activity of AEPF was confirmed *via* CRS--induced gastric ulcers. A potent antiulcer activity of AEPF in rat gastric mucosa was also evidenced.

In present study, in Cold restraint stress induced ulcer model blood parameters such as Glucose, cholesterol and Triglycerides are estimated. The significant increase in blood glucose level was observed because; under stressful conditions adrenal cortex secretes Cortisol in man and corticosterone in rats. Hypersecretion of Cortisol helps in maintenance of internal homeostasis through the process of gluconeogenesis and lipogenesis. Pretreatment with the *AEPF* as well as reference standard drug significantly reduced the elevated glucose levels indicating their suppressant effect on hyper activity of adrenal cortex and maintained the homeostatic mechanism [15].

CONCLUSION

The Aqueous Extract of *Piper nigrum* and *Ferula foetida* at a dose of 400 mg/kg has significantly reduced the incidence of ulcers. The experimental results suggest that the Aqueous Extract of *Piper nigrum* and *Ferula foetida* has significant antiulcer activity.

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