Journal of Chronotherapy and Drug Delivery

ORIGINAL RESEARCH PAPER

Evaluation of Antiulcer Activity of Famotidine-loaded Microemulsion on Experimental Animals

Sajal Kumar Jha[1]*, Roopa Karki[2]

[1] Department of Pharmaceutics, Bengal College of Pharmaceutical Sciences and Research, Durgapur (India)
[2] Department of Pharmaceutics, Acharya BM Reddy College of Pharmacy, Bangalore (India)

* For correspondence

Email: sajal.kumar.jha@gmail.com

Key words
Famotidine, Pylorus ligation, ethanol induced, ulcer score, ulcer index, Microemulsion

Abstract
In the present study famotidine microemulsion was investigated for its antiulcer activity in rats. Ulcers were produced in rats by pylorus ligation method and ethanol induced ulcer in rats. The animals were divided separately for both experiments. In each method animals were divided into three groups of six animals each. Group I served as normal control that received only normal saline. Group II received standard drug famotidine 6 mg/kg orally. Group III received famotidine formulation respectively with a dose equivalent to famotidine 6 mg/kg as microemulsion. The antiulcer activity of pylorus ligation and ethanol induced animals were correlated for the reduction in ulcer levels. Parameters like pH, total acid and ulcer index were calculated and was concluded that the group received famotidine formulation exhibited significant antiulcer activity by both methods as compared to standard drug famotidine. The mean pH, mean total acid, ulcer index and percent ulcer protection for famotidine formulation treated group by pylorus ligation was calculated as: 3.11±0.07, 62.35±4.43 m Eq L-1, 1.54±0.63 and 91.67% respectively. The ulcer index and percent ulcer protection for ethanol induced ulcer model for famotidine formulation was found to be 2±0.63 and 84.62%.From the results it may be concluded that famotidine formulation exhibited significant antiulcer effect which suggests the suitability of microemulsion as a potential drug delivery vehicle for delivery of antiulcer drugs.

J Chronother Drug Deliv | Vol 5 | Issue 1 | 2014: 19-25

INTRODUCTION

Peptic ulcer comprises of heterogeneous disorders, which manifest as a break in the lining of the gastrointestinal mucosa bathed by acid and pepsin. It is the most predominant of the gastrointestinal diseases with a worldwide prevalence of about 40%in the developed countries and 80% in the developing countries. [1] It is generally recognized that peptic ulcer is caused by a lack of equilibrium between the gastric aggressive factors and the mucosal defensive factors. [2] Based on site of attack, peptic ulcer may be classified as oesophageal, duodenal, or gastric. The etiology of gastroduodenal ulcers is influenced by various aggressive and defensive factors such as acid-pepsin secretion, parietal cell, mucosal barrier, mucus secretion, blood flow, cellular regeneration and endogenous protective agents (prostaglandins and epidermal growth factors). [3]

Several orthodox pharmaceutical drugs such as anticholinergic drugs, histamine H2-receptor antagonists, antacids, and more recently, proton-pump inhibitors have been employed in the management of peptic ulcers. Even though wide range of drugs available for the treatment of ulcer, may do not fulfill the requirements and have many side effects such as arrhythmias, impotence and hemopoietic changes are noted. H2 antagonists unlike anticholinergics they do not delay gastric emptying time which may reflexly stimulate gastric secretion because of food remaining in the stomach for long time. Also it does not cause abdominal colic and diarrhea caused by proton pump inhibitors. In recent years large advance in chemical and pharmacological studies has contributed to the knowledge about new therapeutically active compounds and control drug delivery systems for peptic ulcer. Out of the available category of drugs for the treatment of ulcer, H2 antagonist's class of drugs like Famotidine and Ranitidine considered to be the safest drugs available. [4] Hence, this drug has promising future if controlled release formulations are made. Famotidine is *N*′-(amino sulfonyl)- 3-[[[2-[(diaminemethylene) amino]-4-thiazolyl] methyl] thio] propanimidamide a model BCS Class-III drug. It is a potent H2 receptor antagonist used to treat peptic ulcer and hence effectively heals gastric and duodenal ulcers and is also effective in Zollinger-Ellison Syndrome. Theoretical bioavailability of famotidine is 40-50% [5] and the extent of drug release is also shorter which requires repeated dose administration that leads to increased adverse effect. In order to overcome these problems an attempt was made to develop microemulsion drug delivery system for famotidine.

MATERIALS AND METHODS

Famotidine was obtained from Micro Labs (Bangalore, India) as free gift sample. All other chemicals used in this study were obtained commercially and were of analytical (AR) grade.

Animals

Swiss albino rats weighing 150-200 g of either sex were used for this experiment, were selected at random from animal house of the Pinnacle Biomedical Research Institute (PBRI), Bhopal. Institutional animal ethics committee approved the experimental protocol; animals were maintained under standard conditions in an animal house approved by committee for the purpose of control and supervision on experiments on animals (CPCSEA). All animal experiments were approved by Institutional Animal Ethics Committee (IAEC) of PBRI (Regd. No. 1283/C/09/CPCSEA) with protocol approval reference number PBRI/IAEC/11/PN-144. The animals were housed in polypropylene cages and maintained at 24°C ± 2°C under 12 h light/ dark cycle and were feed *ad libitum* with standard pellet diet (Golden feed, New Delhi) and had free access to water.

Pylorus Ligation Induced Ulcer Model

Swiss Albino rats of either sex weighing between (150-200 g) were divided into three groups each of 6 animals. In this method albino rats are fasted in individual cages for 24 h. Test drug or standard drug or control vehicle were administered 30 min prior to pyloric ligation. Group I served as normal control in which normal saline was administered orally; Group II received famotidine 6 mg/kg orally and it was considered as standard; Group III served as famotidine formulation group and the dose equivalent to famotidine 6 mg/kg was administered. The pH was estimated by using pH strips with a pH range of 2-4.5 with a difference range of 0.5. Under light ether anaesthesia, the abdomen was opened and the pylorus was ligated. The abdomen was then sutured. At the end of 4 h after ligation, the animals were sacrificed

with excess of anaesthetic ether, [6] the stomach was dissected out, gastric juice is collected were drained into tubes and were centrifuged at 1000 rpm for 10 min and the volume was noted. The pH of gastric juice is recorded by pH meter. Then the contents are subjected to analysis for total acidity. The stomachs are then washed with running water to see for ulcers in the glandular portion of the stomach. The numbers of ulcers per stomach are noted and severity of the ulcers scored microscopically with the help of 10x lens and each spot was given a severity rating of 1-4 scale as given in Table 1: [7]

Table 1 Scale for grading the severity of ulceration

Ulcer Score	Inference
0	Normal
1	Red coloration
2	Spot ulcers
3	Hemorrhagic streaks
4	Ulcers > 3 but < 5
5	Ulcers > 5

Estimation of Total Acidity

1 mL of supernatant was diluted to 10 mL of distilled water. The solution was titrated against the 0.05 mL/L NaOH using phenolphthalein as an indicator. Titration was continued until the color changed to light pink. The volume of NaOH required was noted and was taken as corresponding to the total acidity. Acidity was expressed as: [4]

$$Total\ Acidity = \frac{[Vol\ of\ NaOH\ \times\ Normality \times 100]}{0.1}\ mEq/L$$

Determination of Ulcer Index

The tracing of the stomach boundary and the ulcerated area on the transparent film was placed on top of a graph paper. The total surface area of the stomach and the lesions was determined in mm^2 from the graph paper. The ratio of total surface area and the total ulcerated area was determined and scoring of the ulcer index was done accordingly. Percentage protection was calculated in the drug treated groups against control using the formula:

$$Percent\ Inhibition = \frac{[UI\ Control - UI\ Treated]}{UI\ Control} \times 100$$

Ethanol Induced Ulcer Model

Swiss Albino rats of either sex weighing between (150-200 g) were divided into three groups with each group consisting of six animals. The animals were fasted for 24 h with free access water. Group I served as normal control, in which normal saline was administered orally; Group II received famotidine 6 mg/kg orally and it was considered as standard; Group III served as famotidine formulation group and the dose equivalent to famotidine 6 mg/kg was administered. Animals were given test drugs or standard drug. One hour later, 1 mL/200 g of 99.80% alcohol was administered orally to each animal. The animals were anaesthetized 1 h later with ether and the stomach was incised along the greater curvature and ulceration was scored. The number of ulcers and the length of each ulcer were measured. Ulcer index was calculated using severity scores and average number of ulcers per animal. [8]

Statistical Analysis

Data are presented as mean ± SEM (standard error of the mean) and n represents the number of rats used for a particular experiment. Comparisons were made between treated and control groups using one-way analysis of variance (ANOVA) followed by Dunnett's test and significance of difference was accepted at $p<0.05$.

RESULTS AND DISCUSSION

Pylorus Ligation-induced Ulceration

Pylorus ligation causes ulceration mainly via increased accumulation of gastric acid and pepsin leading to auto digestion of gastric mucosa. The pH and total acidity levels in the gastric juice of pylorus-ligated rats were significantly decreased by the standard drug, i.e. famotidine (group II), as compared to the saline-treated control (group I) shown in Table 2, thus exhibiting anti-secretory mechanism involved in the anti-ulcerogenic activity through H2 receptors. Ulcer index parameter was used for the evaluation of antiulcer activity since ulcer formation is directly related to the factors such as total acidity. The ulcer index in pylorus ligated control animals was (UI = 12±0.77). The reduction in ulcer index was observed in standard (UI = 5.83±1.07) and in test formulation (UI = 1.54±0.63) were significant ($p<0.05$) when compared to control.

The rats treated with famotidine formulation (6 mg/kg) (group III) exhibited significant ($p<0.05$) decrease in total acidity, whereas, pH was significantly increased when compared with saline-treated control group (Group-I) ($p<0.05$). The famotidine formulation had decreased the gastric acidity induced by pyloric ligation and shown inhibition of 91.67% when compared to control, whereas, standard famotidine exhibited 51.42% of inhibition when compared to control. In control group, the ulcer incidence was 100% (Table 2, Fig 1 and 2).

Table 2. Antiulcer activity of famotidine formulation on pylorus ligation induced gastric ulcer in rats

Groups	Treatment	Dose#	Mean pH	Mean total acid	Ulcer index	Protection (%)
Group I	Normal saline	10	1.96±0.19	104.12±1.84	12±0.77	---
Group II	Famotidine	6	3.01±0.09	66.41±3.89	5.83±1.07	51.42***
Group III	Formulation	6	3.11±0.07	62.34±4.43	1.54±0.63	91.67***

mL/kg or mg/kg; Values are Mean ± SEM; n=6; ***P<0.05 Vs control

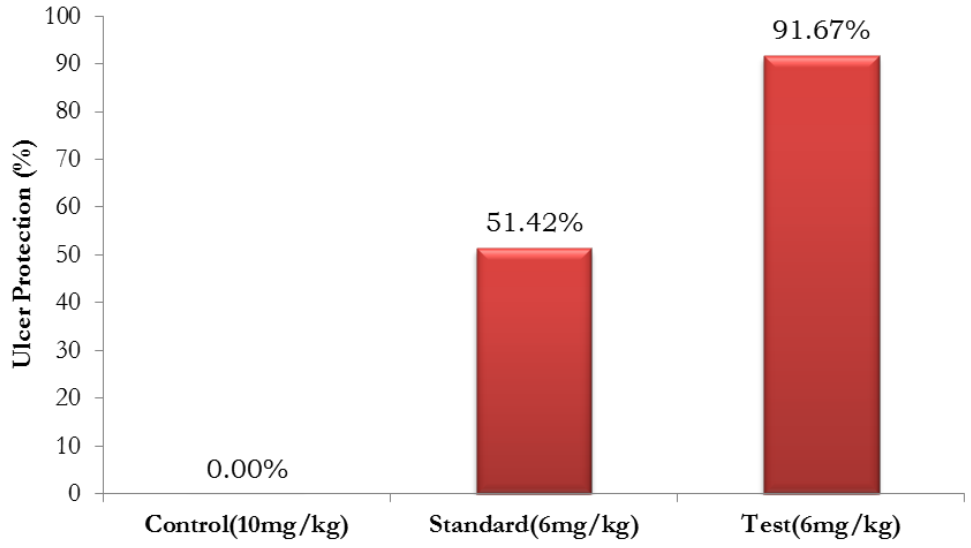
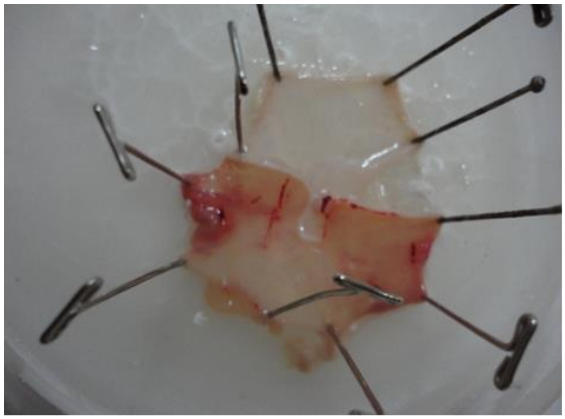
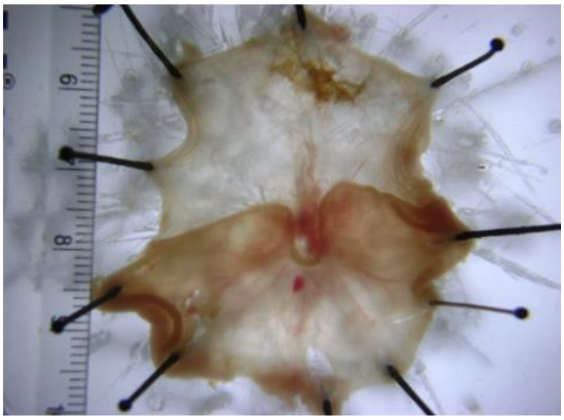


Fig 1. Percentage ulcer protection by standard drug and microemulsion formulation in pylorus ligation-induced gastric ulcer



a) Control (10 mL/kg saline)



b) Standard (6 mg/kg famotidine)

c) Test (6 mg/kg microemulsion)

Fig 2. Photographs of stomach: pylorus ligation-induced gastric ulcers in different groups of treated rats

Ethanol Induced Ulceration

Ethanol is considered a risk factor for developing gastric ulcers. It readily penetrates the gastric mucosa due to its ability to solubilize the protective mucous and expose the mucosa to the proteolytic and hydrolytic actions of hydrochloric acid and pepsin, causing damage to the membrane. The ulcer index in ethanol induced control animals was (UI=13±1.0). The reduction in ulcer index was observed in standard (UI=6±0.77) & in test formulation (UI=2±0.63) were significant ($p<0.05$) when compared to control. Similarly, famotidine formulation has significantly reduced mucosal damage (84.62%) as compared to standard famotidine (53.85%) induced by ethanol, which suggests that famotidine formulation strengthens and protects the gastric mucosal barrier (Table 3, Fig 3 and 4).

Table 3. Antiulcer activity of famotidine formulation on ethanol-induced gastric ulcer in rats

Groups	Treatment	Dose#	Ulcer index	Protection (%)
Group I	Normal saline	10	13±1.0	---
Group II	Famotidine	6	6±0.77	53.85***
Group III	Formulation	6	2±0.63	84.62***

mL/kg or mg/kg; Values are Mean ± SEM; n=6; ***P<0.05 Vs control

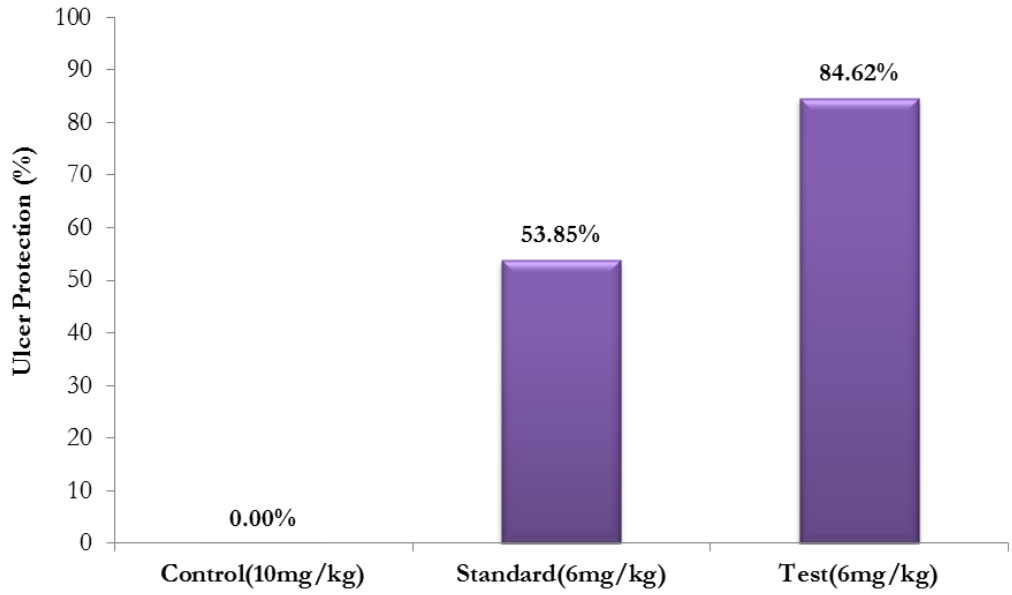
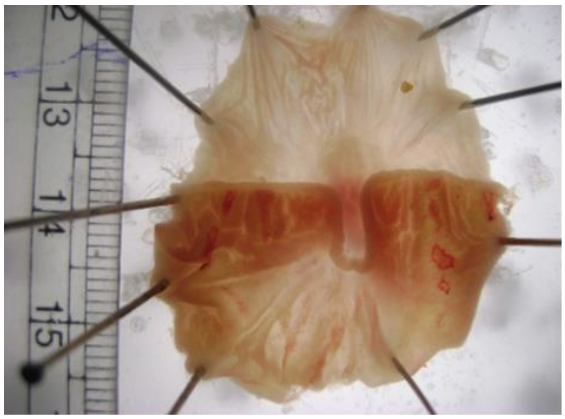
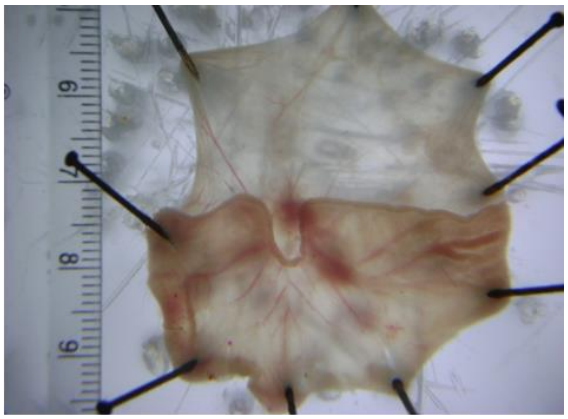


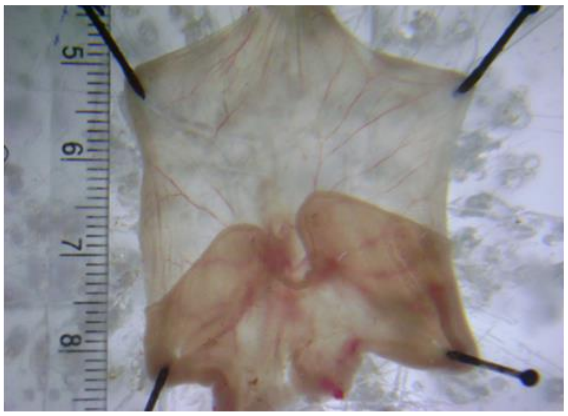
Fig 3. Percentage ulcer protection by standard drug and microemulsion formulation in ethanol-induced gastric ulcer



a) Control (10 mL/kg saline)



b) Standard (6 mg/kg famotidine)



c) Test (6 mg/kg microemulsion)

Fig 4. Photographs of stomach: ethanol-induced gastric ulcers in different groups of treated rats

CONCLUSION

From the present study, it can be concluded that the famotidine loaded microemulsion system could be used as a potential antiulcer agent for the treatment of duodenal and gastric ulcers.

ACKNOWLEDGEMENTS

The authors are grateful to Micro Labs, Bangalore (India) for their kind gift samples of the drug, Famotidine. The authors are also thankful to Pinnacle Biomedical Research institute (PBRI), Bhopal for their assistance in animal studies.

DECLARATION OF INTEREST

It is hereby declared that this paper does not have any conflict of interest.

REFERENCES

1. Goyal RK. Elements of Pharmacology. New Delhi: BS Shah Prakashan; 2008.
2. Rao CV, Sairam K, Goel RK. Experimental evaluation of Bocopa monniera on rat gastric ulceration and secretion. Indian J Physiol Pharmacol. 2000; 44(4): 435-441.
3. Valle DL. Peptic ulcer diseases and related disorders. In: Braunwald E, Fauci AS, Kasper DL, Hauser SL, Longo DL, Jameson JL, editors. Harrison's Principles of Internal Medicine. New York: McGraw Hill; 2005. p. 1746–1762.
4. Ramchandran S, Poovi G, Dhanaraju MD. Evaluation of gastric and duodenal antiulcer activity of famotidine formulation in experimental animals. J Pharmacol Toxicol. 2011; 6(2): 189-195.
5. Rang HP, Dale MM, Ritter JM, Flower RJ. Rang and Dales' Pharmacology. 6th ed. London: Elsevier; 2007.
6. Shay H, Komarov 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.
7. Sharma R, Singh M, Kasture SB, Pooja, Samanta KC. Evaluation of Antiulcer Activity of Leaf Extracts of Laurus nobilis Linn. J Chronother Drug Deliv. 2012; 3(3): 99-107.
8. Hollander D, Tarnawski A, Krause WJ, Gergely H. Protective effect of sucralfate against alcohol-induced gastric mucosal injury in the rat: macroscopic, histologic, ultrastructural, and functional time sequence analysis. Gastroenterology.1985; 88(1):366-374.

Received: Mar 29, 2014; Accepted: Apr 17, 2014

This page is intentionally left blank