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Evaluation of Gastric and Duodenal Antiulcer Activity of Famotidine Formulation in Experimental Animals

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ABSTRACT

In the present study controlled release formulation of famotidine was investigated for its gastric and duodenal antiulcer activity in rats. Ulcers were produced in rats by pyloric ligation method and aspirin induced ulcer in rats. The animals were divided separately for both experiments. In each method animals were divided into four groups of six animals each. Group 1 served as normal control that received only distilled water. Group II served as disease control in which the animals were maintained under same environmental conditions but surgical manipulations done like other groups. Group III received standard drug famotidine 12 mg kg^{-1} orally. Group IV received famotidine formulation respectively with a dose equivalent to Famotidine 12 mg kg^{-1} orally as suspension. The antiulcer activity of pyloric ligated and aspirin induced animals were correlated for the reduction in ulcer levels. Parameters like volume of gastric secretion, 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 stomach biopsy of all the groups were analyzed and was found that rats which received famotidine formulation and standard famotidine showed good healing of ulcers when compared to disease control group of animals. The mean volume of gastric secretions, mean pH mean total acid and ulcer index for famotidine formulation treated group was calculated as 2.76 mL, 5.65, 112 mEq L^{-1} and 1.68, respectively. From the results it can be concluded that famotidine formulation exhibited significant antiulcer effect and the histopathology report also supports and confirm its effect.

Key words: Famotidine, pyloric ligation, total acid, ulcer index, stomach biopsy

INTRODUCTION

For over a century peptic ulcer has been one of the leading causes of gastrointestinal surgery, with high morbidity and mortality rates. The prevalence of gastrointestinal ulcers differs around the world, duodenal ulcers are dominant in the western populations and gastric ulcers are more frequent in Asia especially in Japan. As the prevalence of this disease increases over time, one would expect peptic ulcers to continue to have a significant global impact in the basic health and economic systems and in patient's life quality (Yuan *et al*., 2006).

Peptic ulcers are deep gastrointestinal erosion disorder that involves the entire mucosal thickness, penetrating the muscular mucosa (Tarnawski *et al*., 2005). For decades it was believed

that gastro intestinal ulcerations were caused by the excessive secretion of gastric acid, but many patients presenting such ulcerations had normal acid secretion rates (Wallace and Granger, 1996). Then the researchers reported that peptic ulcers were been caused by an imbalance between the aggressive factors and a number of known defence mechanisms. Exogenous aggressive factors like smoke, anti-inflammatory drugs, alcohol, psychological stress, fatty foods and *Helicobacter pylori* infections triggered tissue necrosis (Barenguer *et al*., 2006) through mucosal ischemia, free radical generation and cessation of nutrient delivery, hydrochloric acid together with pepsin, pancreatic enzymes and bile decreases the defence mechanisms of gastrointestinal mucosa such as the intercellular junctions, local blood flow, mucus/bicarbonate secretion and cellular growth (Kaunitz and Akiba, 2004; Bandyopadhyay *et al*., 2001). In recent years large advance in chemical and pharmacological studies has contributed to the knowledge about new therapeutically active compounds and controlled drug delivery systems for peptic ulcers. Pyloric ligation method (Shay *et al*., 1945) and Aspirin induced gastric and duodenal ulcers are the most recommended method for evaluating antiulcer activities of drugs. Aspirin is one of the most widely used NSAID damages gastrointestinal mucosa by irritant action, causing alteration in mucosal permeability or suppression of Prostaglandin synthesis (Ajitha and Rajanapayana, 2001).

The drugs used in the treatment of ulcer include receptor blockers, proton pump inhibitors, drugs affecting mucosal barrier and act on the central nervous system (Manonmani *et al*., 1995). Even though wide range of drugs available for the treatment of ulcer, many do not fulfill the requirements and have many side effects such as arrhythmias, impotence and hemopoietic changes are noted (Ariyoshi *et al*., 1986). H_2 antogonists unlike anticholinergics they do not cause side effects like dry mouth, urinary retention etc. 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 (Goyal, 2008). Out of the available category of drugs for the treatment of ulcer, H_2 antagonists class of drugs like Famotidine, Ranitidine are considered to be the safest drugs available, Hence this drug has promising future if controlled release formulations are made.

Famotidine is a H_2 receptor antagonist that inhibits acid production by reversibly competing with histamine for binding with H_2 receptors that is located at the basolateral membrane of the parietal cells (Bruntan *et al*., 2006). H_2 receptor antagonists not only inhibit acid secretion induced by histamine, gastrin and cholinergic stimulation, they also promote healing of the duodenal ulcers (Sharma, 2007). Theoretical bio availability of famotidine is 50% (Rang *et al*., 2007) 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 prepare a controlled drug delivery system for famotidine and its pathological influence on stomach was also studied.

MATERIALS AND METHODS

The pure famotidine was procured as a gift sample from Novartis, Bombay. Topfers reagent and sodium hydroxide were procured from Merck, Mumbai. Wistar albino rats of either sex weighing 150-175 g were procured from National Institute of Nutrition and Science (NINS), Hyderabad.

Famotidine was procured as a gift sample from Novartis, Bombay in the year 2009. Topfers reagent and Sodium hydroxide were procured from Merck, Mumbai in the year 2009. Wistar albino

rats of either sex weighing 150-175 g were procured from National Institute of Nutrition and science in the month of February 2010, Hyderabad after getting approval from Institutional animal ethical committee under the guidelines recommended by CPCSEA, New delhi.

Pyloric ligation: Wistar albino rats of either sex were grouped into eight each containing 6 animals. They were kept in the animal house at room temperature 25+2°C, with relative humidity of 45-55% maintained under 12 h light and dark cycle and were fed with standard rat feed and were acclimatized for a week before the study (Bhave *et al.*, 2006; Kath and Gupta, 2006). Group I served as normal control in which distilled water was administered orally in which no pyloric ligation was done, group II served as disease control, group III received Famotidine 12 mg kg^{-1} orally and it was considered as standard, group IV served as Famotidine Formulation group and the dose equivalent to famotidine 12 mg kg^{-1} was administered.

Pyloric ligation was performed for Group II, III and IV as described by Shay *et al.* (1945). Rats were fasted for 36 h prior to the surgical procedure and kept in raised mesh-bottomed cages to avoid coprophagy. Under ether anesthesia the abdomen was opened by a small midline incision below the xiphoid process. The pyloric portion of the stomach was identified, slightly lifted, avoiding traction to the pylorus or damage to the blood supply. The stomach was then replaced carefully and the abdominal wall closed by interrupted sutures. Animals were deprived of both food and water during the post operative period and were sacrificed at the end of 19-20 h after the operation. The stomach was dissected out as a whole by passing a ligature at the esophageal end.

The stomach was separated from the surrounding tissues and organs and thus brought out as a whole along with its contents. The contents were subjected to centrifugation (3000 rpm for 10 min) and then analyzed for mean volume of gastric secretion, mean pH and mean total acid (Ramachandran *et al.*, 2008). The pH was estimated by using indikrom pH strips (Glaxo India Limited, India) with pH ranges of 2-4.5 and 5-8.5 with a difference range of 0.5. Free acidity and total acidity were estimated by titrating 1 mL of centrifuged sample with 0.01 N NaoH, using Topfers reagent as indicator and phenolphthalein indicator respectively. Acidity was expressed in clinical units that are the amount of 0.01 N NaoH base required to titrate 100 mL of gastric secretion (Kulkarni, 1985).

Acidity was expressed as:

$$\text{Total acidity} = \frac{\text{Volume of NaOH} \times \text{Normality} \times 100}{0.1}\ \text{mEq L}^{-1}$$

Aspirin induced ulcer: In Aspirin induced ulcer models (Hegde *et al.*, 1996) four groups of albino rats of either sex weighing 150-175 g, with each group consisting of 6 animals were used. The first group served as a normal control the second group served as disease control and the third group served as standard group that received famotidine 12 mg kg^{-1} (Sener *et al.*, 2004) and group four received famotidine formulation equivalent to famotidine (Ramachandran *et al.*, 2010) 12 mg kg^{-1}. All the animals received above treatment once daily for eight days orally. After 8 days of treatment, animals were fasted for 24 h. Ulcer was produced by administration of aqueous suspension of aspirin (200 mg kg^{-1} orally) on the day of sacrifice. The animals were sacrificed

4 h later and stomach was opened to calculate the ulcer index by kunchandy method (Kunchandy *et al.*,1985).

(The antiulcer activity was carried out after the ethical approval from CPCSEA and it was done as per the recommended guidelines of CPCSEA reg. No. 1069/AC/07/CPCSEA)].

RESULTS AND DISCUSSION

In aspirin and pylorus ligation induced gastric ulcer models the famotidine formulation reduced the gastric volume, total acidity and ulcer index (Table 1) thus showing the anti secretory mechanism involved in the antiulcerogenic activity (Malairajan *et al.*, 2008) through H_2 receptors.

Ulcer index parameter (Table 2) was used for the evaluation of antiulcer activity since ulcer formation is directly related to the factors such as gastric volume and total acidity (Goel and Bhattacharya, 1991). From the results it is clear that gastric volume, total acidity and ulcer index of formulated famotidine were significantly reduced (Ramachandran *et al.*, 2010) as 2.76±0.24 mL, 5.65±0.51, 112±0.50 mEq L^{-1} and 1.68±0.24, respectively (Subudhi *et al.*, 2009; Yesilada *et al.*, 1997). The data obtained for the formulated famotidine was compared with that of Normal animal's data and it was found that there was complete healing produced denoting the Pharmacological efficiency of the formulation.

The biopsy reports (Tawfeq *et al.*, 2005; Shirwaikar *et al.*, 2006) of all the groups of rats were analyzed and shown in Fig. 1a-d and it was found that the section of stomach from normal control rat showed normal architecture, section of stomach from disease control rat showed severely damaged stomach cells with chronic inflammation, section of stomach from famotidine treated rat showed mild damaged cells and the section of famotidine formulation treated also showed mild damaged cells confirming the antiulcer effect of Formulated famotidine and also there is no evidence of extra tissue damage as seen in the biopsy report.

Table 1: Antiulcer effect of Famotidine formulation on pyloric ligation induced gastric ulcer in rats

	Parameters			
Groups	Mean volume of gastric secretion	Mean pH	Mean total acid	Ulcer index
Control	3.23±0.15	4.56±0.05	94.5±1.02	2.23±0.35
Disease control	5.75±0.28	2.45±0.16	164.2±1.89	5.65±0.21
Standard Famotidine	2.54±0.16**	5.84±0.81***	109±0.05**	1.01±0.08***
Famotidine formulation	2.76±0.24**	5.65±0.51**	112±0.50**	1.68±0.24**

Values are expressed as Mean±SEM, n = 6 in each group. **p<0.01, ***p<0.001

Table 2: Antiulcer effect of Famotidine formulation on aspirin induced gastric ulcer in rats

	Parameters	
Groups	Dose	Ulcer score
Control	Normal saline 2 mL kg^{-1}	2.65±0.52
Disease control	Normal saline 2 mL kg^{-1}	4.84±0.33
Standard famotidine	Famotidine 12 mg kg^{-1}	1.31±0.16**
Famotidine formulation	Formulation equivalent to Famotidine 12 mg kg^{-1}	1.48±0.01**

Values are expressed as Mean±SEM, n = 6 in each group. **p<0.01, ***p<0.001

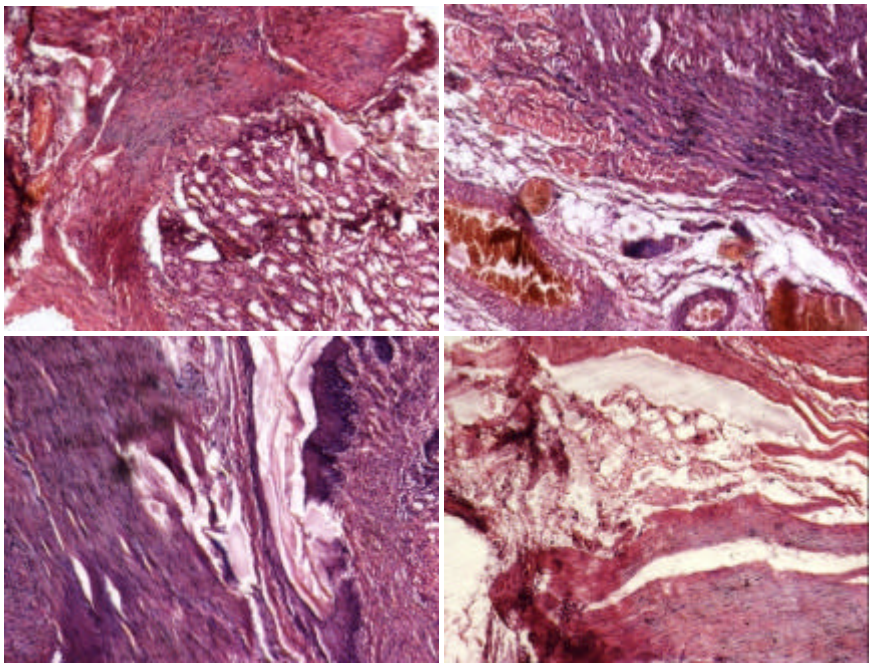

Fig. 1: Biopsy of Rat Stomach induced with ulcer. (a) Section of stomach from normal control rat shows normal architecture, (b) Section of stomach from disease control rat shows severely damaged cells, (c) Section of stomach from famotidine treated rat shows mild damaged cells and (d) Section of stomach from famotidine formulation treated rat shows mild damaged cells

CONCLUSION

Hence it can be concluded that the formulated famotidine preparation could be used as a potential antiulcer agent for the treatment of duodenal and gastric ulcers.

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