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Original Research Article

Evaluation of antiulcer activity of diltiazem in rats

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ABSTRACT

Background: The risk factors for patients with cardiovascular diseases and gastrointestinal diseases overlap. Majority of the patients have both problems coexistent. Thus, there is need of medicine that can be used for both the diseases.
Methods: Rats weighing 150-250gm of either gender were procured for the study from central animal house. The animals were divided into 7 groups. Control group (Distilled water 2ml), diclofenac sodium (12mg/kg), diltiazem (10mg/kg), diltiazem (30mg/kg), diltiazem (60mg/kg), ranitidine (8mg/kg), ranitidine (16mg/kg). After six hours, scarification of animals was done by cervical dislocation. Size of ulcer, number of gastric ulcers, mean gastric irritancy index, ulcer index, and ulcer scoring were the parameters that were studied.
Results: Diltiazem in dose of 10, 30 and 60 mg/kg showed reduction in all parameters in dose dependent manner. Diltiazem (60mg/kg) showed marked reduction in mean diameter of ulcerated surface area (0.46±0.36), number of ulcers (4.10±2.05), size of ulcers (1.07±0.48), total mucosal surface area (7.60±1.38), and total ulcerated surface area (0.263±0.3). Diltiazem (60mg/kg) showed significant reduction of the parameters as compared to other doses of Diltiazem. Also, diltiazem (60mg/kg) was comparable to Ranitidine in all the parameters. Diltiazem (60mg/kg) also showed reduction in average number of ulcers, ulcer index, mean gastric irritancy index and ulcer scoring as compared to diclofenac sodium (12mg/kg).
Conclusions: Diltiazem has shown to have ulcer prevention property; this can be useful in patients having concomitant cardiovascular and gastrointestinal problems.

Keywords: Diclofenac, Gastric ulcer, Peptic ulcer, Ranitidine

INTRODUCTION

In everyday life, peptic ulcer disease affects millions of people throughout the world.[1] Dyspepsia affects 10% to 40% of general population. Peptic Ulcer disease is one of the diseases in which quality of life is significantly impaired.[2] Almost 5-15% of adult population of world are suffering from peptic ulcer disease and its consequences.[3]

In the United States, approximately four million people have duodenal and gastric ulcer while 180,000 patients are hospitalized each year due to peptic ulcer disease. There is 10% likelihood of developing peptic ulcer in about 10% males and 4% females.[1,3] Peptic ulcer incidence in India is 2.9% and for gastric ulcer is 2.7%.[2]

With time and change in life style its incidences have increased significantly.[1] Estimated that on daily basis 50% of healthy individuals experience heartburn symptoms. alcohol, nonsteroidal anti-inflammatory drugs (NSAIDs) and stress are the major causes of peptic ulcer disease.[4] The imbalance between mucosal protecting and damaging mechanisms is responsible for Peptic ulcer. The mucosal damaging mechanisms include acid and pepsin while mucosal protecting mechanisms include mucus, bicarbonate, Prostaglandin E_2 and I_2. Acid secretion is an

important process of the stomach. The pepsinogen activation by gastric acid activates the digestive process that kills bacteria, other microbes thus providing a stable gastric environment.[4,5]

Calcium is critical for stimulus secretion coupling in gut as hypercalcaemia is associated with increased gastric acid secretion. There is calcium dependent release of Histamine, acetyl choline, gastrin and hydrochloric acid. Histamine release is dependent on both extracellular and intracellular calcium, thus suggesting that development of gastric ulcer is dependent on calcium release.[2,4]

Diltiazem, well known cardiovascular drug that having calcium channel blocking property is used clinically for the treatment of hypertension and angina pectoris.[5] Diseases of gastrointestinal disorders and hypertension often coexists, therefore medicines for peptic ulcer diseases and hypertension need to be administered together for cure of gastrointestinal tract disorders and hypertension.[6] Thus a drug that can be used for both hypertension and peptic ulcer disease is the need of the hour.

METHODS

From central animal house Albino rats weighing around 150 to 250gm and from either gender were procured. The animals were randomly divided into 7 groups and all the study were fed standard rat diet with water ad libitum; also, they were placed in different cages having floor with grating to prevent coprophagy.

- Group 1: Control group (Distilled water 2ml)
- Group 2: Diclofenac sodium (12mg/kg)
- Group 3: Diltiazem (10mg/kg) + Diclofenac sodium (12mg/kg)
- Group 4: Diltiazem (30mg/kg) + Diclofenac sodium (12mg/kg)
- Group 5: Diltiazem (60mg/kg) + Diclofenac sodium (12mg/kg)
- Group 6: Ranitidine (8mg/kg) +Diclofenac sodium (12mg/kg)
- Group 7: Ranitidine (16mg/kg) + Diclofenac sodium (12mg/kg)

Table 1: Effect of different doses of diltiazem and ranitidine on diclofenac sodium induced gastric ulcer.

Groups	Drugs administered	Mean diameter of mucosal surface (cm)	Mean diameter ulcerated surface (cm)	No. of ulcers	Size of ulcers (mm)	Total mucosal surface area (cm^2)	Total ulcerated surface area (cm^2)
1	Control	2.91±0.26	-----	-----	-----	6.712±1.18	-----
2	Diclofenac sodium (12mg/kg)	3.087±0.313	1.56±0.645	7.6±2.3	2.012±0.327	7.56±1.51	2.198±1.88
3	Diltiazem (10mg/kg)	2.8±0.54	0.972±0.532	6.875±1.885	1.4±0.325	6.245±1.574 ***	0.937±0.799
4	Diltiazem (30mg/kg)	3.06±0.302	0.80±0.443	5.25±1.2817	1.5±0.521	7.43±1.455***	0.599±0.4811*
5	Diltiazem (60mg/kg)	3.1±0.287	0.468 ± 0.365**	4.10±2.05*	1.075±0.486*	7.60 ±1.38**	0.263±0.323**
6	Ranitidine (8mg/kg)	2.912±0.356	0.1225±0.149*** #	1.785±0.834***###$	0.79±0.74**	6.752±1.65	0.026±0.046**
7	Ranitidine (16mg/kg)	3.1±0.37	0.016±0.031***##$	1.375± 0.744***### #$$	0.412±0.210***#$	7.645±1.768### #$$$^	0.0087±0.0018**

(* significant as compared to group 2), (#significant as compared to group 3), ($ significant as compared to group 4), (^ significant as compared to group 5), (§significant as compared to group 6)

Drugs and preparations

Solutions of diltiazem (Usan Pharmaceuticals Pvt. Ltd.) indomethacin (National Pharmaceuticals Pvt. Ltd.) were freshly prepared in distilled water separately. Ethanol 80% (Global Chemicals) and ranitidine ampoules (Johnlee Pharma Ltd.) were the other drug preparations used for the study. Diltiazem solutions were prepared in distilled water. The doses of diltiazem used were 10mg/kg, 30mg/kg and 60mg/kg given intraperitonially (i.p.).[7]

Ulceration in Rats was induced using diclofenac sodium, the diclofenac solutions were prepared fresh in distilled water and administered orally. The animals were divided into 07 groups randomly and there were 08 animals in each group. Prior to experiment animals were fasted for 24 hours, only water was allowed. The control group was given (2ml) distilled water i.p. 30 min before diclofenac sodium (12mg/kg). The test group received diltiazem (10, 30 and 50mg/kg i.p) 30 min before diclofenac (12mg/kg). Similarly, in standard group ranitidine was given (8 mg/kg and 16mg/kg) 30 min before diclofenac sodium (12mg/kg). After six hours, the animals underwent scarification by cervical dislocation, following this the abdomen was opened by midline incision that was taken below xiphoid process. The stomachs of animals were removed with minimal impact and opened along the greater curvature. After opening stomachs were pinned

with mucosal surface facing upwards for examination. The cardiac and pyloric ends were ligated, a small nick was taken on the fundus of stomach and stomach was opened along the greater curvature. The parameters of the study were calculated by observing under magnifying glass.

Table 2: Comparison of effects of different doses of diltiazem (10,30,60mg/kg) and ranitidine (8,16mg/kg) on different parameters.

Groups	Drugs administered	Average no of ulcers	Ulcer index	Mean gastric irritancy index (mm)	Ulcer scoring
2	Diclofenac sodium (12 mg/kg)	7.6	2.9	15.89	22
3	Diltiazem (10 mg/kg)	5.87	1.5	9.619	16
4	Diltiazem (30 mg/kg)	3.25	0.806	7.875	17
5	Diltiazem (60 mg/kg)	2.13	0.346	4.413	13
6	Ranitidine (8 mg/kg)	0.785	0.18	1.410	09
7	Ranitidine (16 mg/kg)	0.375	0.013	0.566	03

The following parameters were studied:

Size of ulcer

Each lesion is measured along its greatest length. In case of Petechia five of these are considered equivalent to 1mm ulcer size. The instrument used for measuring was Digital Vernier Calliper.

Number of gastric ulcers

Gastric ulcers were measured on the surface of mucosal surface of the stomach

Mean gastric irritancy index

It was calculated by multiplying mean ulcer number with mean gastric ulcer size. Ulcer index was measured by following formula.[8]

The Formula is 10/X (where X= Total mucosal area/Total ulcerated area)

Using digital Vernier calliper

The diameter of mucosal surface and total mucosal surface area was calculated. To calculate the total ulcerated area, the diameter of ulcerated area was calculated by multiplying the ulcer size and number of ulcers.

Ulcer scoring

Scoring used was as follows: If ulcer size <1mm than score of 01, 1-1.7mm than score of 02, 1.8-2.5mm than score of 03 and if >2.5mm than score of 04.

Statistical analysis

Data in the study are expressed as mean±standard deviation. For the study Unpaired 't' test and One-way ANOVA test was used followed by posthoc Bonferroni's test considering $P<0.05$ to be statistically significant. For statistical analysis of the data Graphpad software having 5.1 version was used.

RESULTS

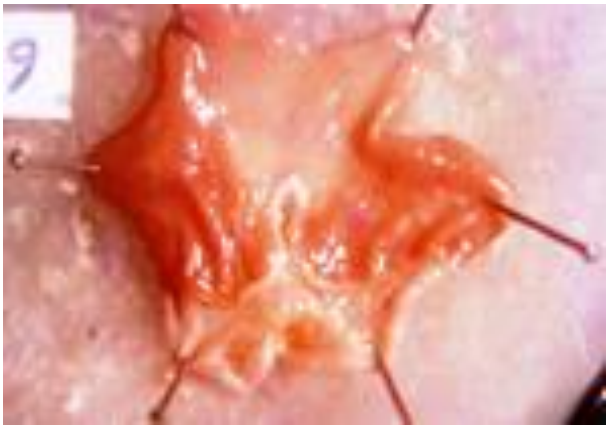

Figure 1: Rat stomach in Group 1.

Figure 2: Rat stomach in Group 2.

Figure 3: Rat stomach in Group 3.

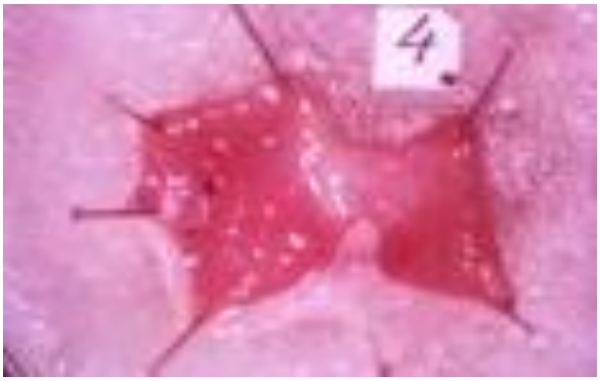

Figure 4: Rat stomach in Group 4.

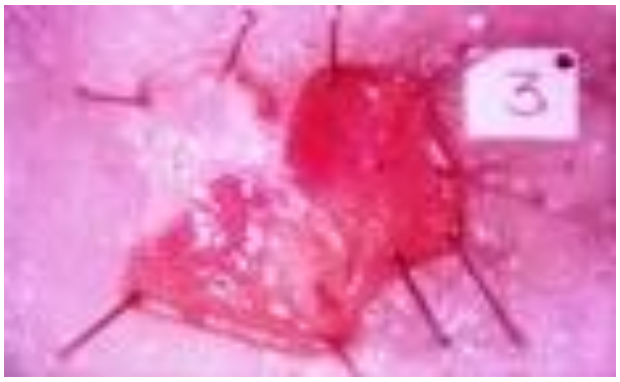

Figure 5: Rat stomach in Group 5.

Figure 6: Rat stomach in Group 6.

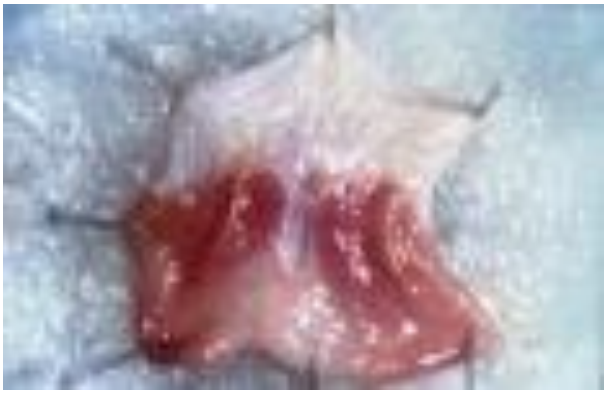

Figure 7: Rat stomach in Group 7.

Effect of different doses of diltiazem and ranitidine on diclofenac sodium induced gastric ulcer is shown in Table 1 and comparison of effects of different doses of diltiazem (10,30,60mg/kg) and ranitidine (8,16mg/kg) on various parameters is shown in table 2. Rat stomach treated with distilled water 2ml given i.p. is shown in Figure 1. Rat stomach given diclofenac sodium 12mg/kg is shown in Figure 2. Figure 2 shows ulcer over the entire gastric ulcer.

Rat stomach treated with diltiazem (10mg/kg), diltiazem (30mg/kg), diltiazem (60mg/kg) is shown in Figure 3, 4 and 5 respectively. Figure 3 shows slight reduction in size, number and severity of ulcers. Figure 4 shows reduction in size, number and severity of ulcers. Figure 5 shows significant reduction in size and number of ulcers. Rat stomach treated with ranitidine (8mg/kg) and ranitidine (16mg/kg) is shown in figure 6 and 7 respectively. Figure 6 shows negligible gastric ulcers. Figure 7 shows total absence of gastric ulcers.

DISCUSSION

Hypertension and peptic ulcer disease are the two diseases that often co-exist especially in younger population. For both the diseases stress, diet and personality type are the aggravating factors.[9] Calcium channel blocking agents are the medicines that are commonly used in the management of cardiovascular diseases including hypertension, angina and myocardial infarction.[10]

Calcium ions are considered to play crucial role development of different ulcers. The important physiological functions of stomach i.e. secretion of gastric acid and release of gastrin release are calcium dependent.[11] Calcium channel blockers have the property to interrupt the calcium conduction and thus halt the action. CCB increase total carbohydrate and protein content ratio of gastric juice, thus strengthening the mucosal defence. CCB also cause increase in production of mucus. Other actions like upregulation of the nitric oxide system and mast-cell degranulation inhibition are said to provide protection to gastric mucosa.[9] Dihydropyridine CCB are reported to enhance production of NO whereas as verapamil did not

have any effect on NO in vitro.[12] Diltiazem is a calcium channel blocker of non-dihydropyridine type.[5]

In present study, diltiazem has the property of ulcer preventing activity in diclofenac induced gastric ulcers. Different parameters in present study were used like number of gastric ulcers gives clear indication of ulcer inducing property of diclofenac and protective effect of diltiazem. Also, ulcer index is important for judging the severity of lesions. The mean gastric irritancy index is useful for studying the dose dependent ulcerogenecity of NSAIDS as well as the effect of ulcer protective agents.

Blockers of calcium channel relax smooth muscles by inhibiting the influx of calcium through the cell membrane. The important inhibitory effects shown by these substances on cardiovascular smooth muscle suggest the possibility of a similar action on the smooth muscle and gastrointestinal tract. In addition, contraction is not the only physiological function in which fluxes of calcium play an important role in cell regulation. Gastric acid secretion has been shown to be dependent on calcium entry and can be altered by agents that inhibit calcium entry. In model of ligation of pylorus rats there was dose dependent reduction in volume of gastric acid secretion.[13] The influx of calcium resulting due to gastric stimulation can be blocked by calcium antagonist and not by antihistamines. Calcium channel antagonists block the parietal cell hydrogen potassium ATPase that causes reduction in acid secretion.[14] Reduction of calcium concentration in the nutrient solution modifies the responses to Ach, gastrin and histamine in canine isolated parietal cells.[7]

Study by Mandal had shown that the antiulcer and anti-secretory effects of famotidine was potentiated by nifedipine, verapamil and diltiazem.[15] Diltiazem, nitrendipine, and verapamil were the calcium channel blockers that reduced the gastric lesions in resistant to cold stress model.[14] Nifedipine and diltiazem in dose dependent mode provided protection in indomethacin, pyloric ligation and absolute ethanol-induced ulceration models of rats.[9] While there were contrary study by Kadalmani had shown that in acute study, the animals belonging to diltiazem group did not show any significant ulcer protecting effect while chronic study had shown significant improvement in the ulcer index compared to the other CCB.[11]

Ranitidine was used, it has the property of safety, efficacy and an ideal comparator to effect of ulcer protective agents in an experimental drug.[9] Study by Jain had showed that verapamil and diltiazem showed reduction in volume of gastric secretion without affecting pepsin activity of gastric juice.[7]

Influx of transmembrane calcium is responsible for blocking the serotonin, histamine release from stomach wall mast cell. This combined effect on histaminergic, gastrinergic and cholinergic results in reduced stimulation of hydrogen potassium ATPase exchange pump thus reducing hydrochloric acid secretion.[15]

In present study diltiazem demonstrated gastric ulcer prevention property of diclofenac. This property can be useful in further research on patients having cardiac disease and that are prone to develop gastric ulcers or peptic ulcer disease.

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

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