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## EVALUATION OF GASTROPROTECTIVE EFFICACY AND SAFETY PROFILE OF LOW DOSE CIMETIDINE AND L-GLUTAMINE USING GASTRIC AND DUODENAL ULCER MODELS IN RATS

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### ABSTRACT

The long-term use of histamine H<sub>2</sub>-receptor blockers especially cimetidine and ranitidine at recommended doses in peptic ulcer disease is associated with various adverse effects. The dose-dependent nature of these adverse effects calls for the use of low doses of these drugs in combination with an amino acid supplement with cytoprotective activity to reduce the adverse effects, improve compliance and achieve positive outcomes. The present study evaluates the gastroprotective efficacy of a combination of low dose cimetidine with L-glutamine on acutely induced gastric and duodenal lesions using ethanol/hydrochloric acid, water immersion and cysteamine, and chronically induced gastric ulcers using ethanol and indomethacin/pyloric ligation. The effects of long term administration of the drug combination on sperm count/motility and testosterone level were also evaluated. Acute gastric ulcers were induced by intragastric instillation of 0.15 M Hydrochloric acid in 70 % v/v ethanol and water immersion stress in rats while duodenal ulcer was induced using cysteamine. Chronic gastric ulcers, on the other hand, were generated by administration of ethanol (70 % maintained with 20 % ethanol for 6 days) and indomethacin (20 mg/kg) + pyloric ligation. Animals were administered the drugs orally for 21 days (in chronic ethanol-induced) and 7 days (for indomethacin/pyloric ligation). Parameters measured included ulcer indices (severity and score), superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and malondialdehyde (MDA), sperm count, sperm motility, testosterone levels. Oral administration of a combination of L-glutamine (200 mg/kg) and cimetidine (10 mg/kg) caused significant ( $p < 0.01$ ) reductions in the number and severity of ulcerations in all the models of ulcer used, compared to the control. However, no significant changes were observed in sperm count and motility as well as blood testosterone level compared to when cimetidine was used alone. The study showed that a combination of low dose cimetidine with L-glutamine conferred protective effects on acute gastric and duodenal injuries as well as chronic gastric injury produced by ethanol and indomethacin/pyloric ligation in rats. There was, however, no apparent attenuation of the side effects associated with cimetidine based on the parameters measured.

**KEYWORDS:** Cimetidine, L-glutamine, Peptic ulcer, Pyloric ligation, Cysteamine

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### INTRODUCTION

Peptic ulcer is a heterogeneous group of disorders involving the upper gastrointestinal tract that affects millions of people every year [1] with a high rate of morbidity particularly for the population of non-

industrialized countries [2]. In Nigeria, 2.1-6.0 % of the population is affected with gastric ulcer [3].

The pathophysiology of peptic ulcer disease involves an imbalance between aggressive and protective factors in the stomach, such as acid-pepsin secretion,

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mucosal barrier, mucus secretion, blood flow, cellular regeneration, prostaglandins and epidermal growth factors [4, 5, 6]. Hence the integrity of gastroduodenal mucosa is maintained by a homeostatic balance between the aggressive factors and protective factors.

Some of the clinically available drugs for the treatment of peptic ulcer disease, including antacids, anticholinergics, proton pump inhibitors and the H<sub>2</sub>-receptor antagonists [7, 8], have shown incidences of relapse, drug interactions and side effects such as arrhythmias, impotence, gynaecomastia, haematopoietic changes, etc [9].

Cimetidine is an established histamine H<sub>2</sub>-receptor antagonist which inhibits acid secretion by the parietal cell. Despite its high efficacy (more rapid onset of action that makes it useful for patient-directed management of mild or infrequent ulcer symptoms), cimetidine has fallen out of use due to the numerous and significant interactions with other drugs via its inhibition of the cytochrome P-450 enzyme [10]. For example, cimetidine may decrease metabolism of some drugs such as oral contraceptives. Cimetidine also has effect on exogenous testosterone at androgen target tissue (e.g. prostate) by inhibiting the dihydrotestosterone binding to its cytoplasmic receptor, leading to exaggerated effects of estrogens thereby producing hyperprolactinemia along with gynecomastia [11]. In women, this can lead to galactorrhea [12]. These side effects have been shown to be dose-dependent [13]. However, recent findings have demonstrated the anticancer potential of cimetidine on the gastrointestinal tract [14, 15].

In the search for better antiulcer drugs, the concept of combination therapy, being utilized in the management of other diseases, becomes necessary. Since the associated side effects of cimetidine are dose-dependent, it is envisaged that the combination of low dose cimetidine with another agent, with a different mechanism of action will attenuate these side effects while still retaining its therapeutic efficacy.

L-glutamine, a conditionally essential amino acid, has been shown to possess antiulcer properties and to prevent stomach cancer [16]. The role of L-glutamine in gastric mucosal protection had been previously reported for stress-, indomethacin- and aspirin-induced ulcer formation and was thought to elicit protection by buffering acid back-diffusion and acid-

induced mucosal damage [17, 18, 19]. L-glutamine reduces histamine stimulated acid secretion, stimulates the secretion of mucus from the stomach lining and is beneficial in off-setting gastric damage caused by *H. pylori* [20]. Apart from its antiulcer effects, L-glutamine through its ability to increase brain dopamine levels leading to inhibition of prolactin release with a consequent increase in testosterone levels has been reported useful in the treatment of impotence.

To the best of our knowledge, there are no studies on the effects of co-administration of L-glutamine on the efficacy and safety profiles of cimetidine in peptic ulcer disease. The present study, therefore, evaluates the effects of acute and long term administration of low dose cimetidine with glutamine on different types/animal models of ulcer and investigating the possible attenuation of the usual limiting side effects associated with cimetidine by co-administration with L-glutamine

## MATERIALS AND METHODS

### Animals

Sprague-Dawley rats (200±50 g) of both sexes were obtained from the Laboratory Animal Centers of Department of Pharmacology and Toxicology, University of Benin, Benin City, and University of Ibadan, Ibadan Nigeria. The animals maintained under standard diet (Bendel Feeds Ltd. Ewu, Edo State) and water *ad libitum* were fasted overnight with free access to water prior to experiments. Animals were handled according to the protocol outlined in "Principles of Laboratory Animal Care" (National Institute of Health Guide for Care and Use of Laboratory Animals, Pub No. 85 – 23, revised 1985).

### Drugs and Reagents

Cimetidine (Ranbaxy Pharmaceutical Company, India); Cysteamine, L-glutamine and Indomethacin were obtained from Sigma-Aldrich Laborchenikalien, GmbH Germany; Misoprostol (Pharmacia, UK); Omeprazole (Nifan Laboratories Ltd., Lagos, Nigeria); Ranitidine (Glaxo Smith Kline Ltd, UK). Other reagents used were of the analytical grade. Stock solutions of drugs and reagents were prepared, appropriately, prior to the experiment.

## Pharmacological tests

### Ethanol/Hydrochloric acid-induced ulcers

Rats of both sexes were randomly divided into 7 groups of 5 animals per group and starved for 24 h but had free access to water. Water was however withdrawn 2 hours before experiments.

Rats were pretreated orally with cimetidine (10 and 50 mg/kg), L-glutamine (200 and 1000 mg/kg), cimetidine [(10 mg/kg)+ L-glutamine (200 mg/kg)] and misoprostol (50 µg/kg) one hour before intragastric instillation of 0.15M Hydrochloric acid in 70 % v/v ethanol (4 ml/kg) to all the groups [21]. Distilled water was given to animals in the control group. One hour following ethanol/HCl administration, the rats were sacrificed by overdose of ether. The stomach was isolated and opened along the greater curvature. Macroscopic examination of the stomachs of the animals in all the groups was done. The presence of ulcers was counted using a magnifying glass. The diameter of the ulcers was measured by means of vernier calipers and scored on a scale of 0-10 [22]. The ulcer index (UI) was calculated as:

$$UI = U_N + U_S + U_P \times 10^{-1} [23].$$

Where:  $U_N$  = Average number of ulcers per animal

$U_S$  = Average of severity score

$U_P$  = Percentage of animals with ulcers

### Water-immersion stress induced ulcers

Rats were randomly allotted to 7 groups of 5 animals each and starved for 24 hours with access to water *ad libitum*.

Group 1 served as the control and received distilled water (3 ml/kg) orally, groups 2 and 3 received cimetidine (10 and 50 mg/kg, respectively), groups 4 and 5 received L-glutamine (200 and 1000 mg/kg, respectively), group 6 received cimetidine (10 mg/kg) + L-glutamine (200 mg/kg) and group 7 received omeprazole (20 mg/kg). All drug administrations were via the oral route.

One hour following drug administration, the rats were immersed in water (28 °C) contained in a rectangular glass of dimensions 48 cm by 24 cm (water level of 15 cm to prevent drowning of rats). Four hours later, each rat was removed from the water and sacrificed by ether anesthesia. The stomach was isolated, scored for the presence of ulcers [24] and the ulcer

index was calculated according to the method of Vogel et al [23].

### Cysteamine-induced duodenal ulcers

Rats were allotted to 7 groups of 5 rats each and were given the drugs via the oral route. Groups 1 and 2 received 10 and 50mg/kg cimetidine, respectively. Groups 3 and 4 were given L-glutamine at doses of 200 and 1000 mg/kg respectively, while Group 5 received cimetidine (10 mg/kg) plus L-glutamine (200 mg/kg). Distilled water (3 ml/kg) served as control and ranitidine (50mg/kg) was the standard drug. One hour after administration of drugs, ulcer was induced by oral administration of cysteamine hydrochloride (400 mg/kg, 10 % solution in normal saline) two times at four hour intervals [25]. The animals were fed with food and water *ad libitum* throughout the period of experiments and drugs were administered 30 minutes before each dose of cysteamine.

Twenty-four hours after the first dose of cysteamine, the animals were sacrificed by excess ether inhalation. The stomach and duodenum were isolated and assessed for the presence of lesions. Number of ulcers in the duodenum was counted and the intensity of ulcers was scored according to the method of [26], where:

0	=	Normal mucosa
1	=	Superficial mucosal erosion
2	=	Deep ulcer or transmural necrosis
3	=	Perforated or penetrated ulcer.

The ulcer index was calculated as the sum of the arithmetic mean of the intensity in a group and the ratio of positive (number of animals with ulcers)/total number of animals multiplied by 2. Stomach ulcers were scored on a scale of 0-10 [22].

### Ethanol-induced chronic gastric ulcer

The method used was similar to that already described [27]. Male rats received 70 % ethanol on the first day to induce acute ulcer and 20 % ethanol for the subsequent 6 days to maintain the gastric ulcer. Animals were placed in 7 groups of 6 animals each. Group 1 served as the control and received distilled water (3 ml/kg) orally while group 2 received ranitidine (50 mg/kg). Groups 3 and 4 were administered by oral intubation cimetidine at doses of 10 and 50 mg/kg, respectively. Rats in group 5 received L-glutamine (200 mg/kg) while those in groups 6 and 7 were given prophylactically and therapeutically, a combination of cimetidine (10

mg/kg/day) plus L-glutamine (200 mg/kg), respectively.

In the prophylactic administration, treatment was commenced from the first day of ulcer induction while in the therapeutic protocol; treatment was commenced on the 8<sup>th</sup> day and lasted for 14 days. At the end of the administration period, all animals were sacrificed by excess ether inhalation. The stomach was isolated and the presence of ulcers was counted with the aid of a magnifying glass. The diameter of the ulcers was measured using a digital vernier caliper and scored on a scale of 0-6 [28] as follows:

0 = no ulcer

1 = hemorrhagic and slightly dispersed ulcers

2 = pin point erosions

3 = one to five small erosions less than 2mm

4 = more than 5 small erosions less than 2mm

5 = one to three large erosions above 2mm

6 = more than 3 large erosions above 2mm

The ulcer index (UI) was determined according to the method of Vogel *et al.* [23] and the percentage inhibition was calculated as:

$$= \frac{\text{Ulcer index}_{(\text{Control})} - \text{Ulcer index}_{(\text{Treatment})}}{\text{Ulcer index}_{(\text{Control})}} \times 100$$

Blood samples were collected, from rats in the control and test groups, by ocular puncture into EDTA bottles for quantitative determination of testosterone concentration using the enzyme immunoassay technique. The caudal epididymides were also removed for assessment of sperm count and motility [29].

The antioxidant enzymes activities of the stomach tissues were determined spectrophotometrically according to standard methods [30, 31, 32, 33].

### Indomethacin plus pylorus ligation induced ulcer

A modification of the method of Majumder *et al.* [34] was used. Animals were randomly divided into 6 groups of 6 animals each. Group 1 was treated with cimetidine (50 mg/kg/day). Groups 2, 3 and 4 received, L-glutamine (200 mg/kg), ranitidine (50 mg/kg/day) and omeprazole (20 mg/kg/day), respectively. Group 5 was given cimetidine (10mg/kg/day) + L-glutamine (200 mg/kg/day) while distilled water (3 ml/kg/day). All drug administrations were via the oral route and lasted for 7 days, with the last dose of the drugs given 1 hour before oral

administration of a single dose of indomethacin (20 mg/kg). Six hours later, the abdomen was opened by a midline incision, under light ether anaesthesia and pyloric ligation was done without causing any damage to the blood supply [35]. The stomach was replaced carefully and the abdomen was closed with continuous sutures. The animals were deprived of food during the post-operative period. After about 18 hours, the animals were sacrificed with overdose of ether, stomach was dissected and contents were collected into a tube and centrifuged at 2000 rpm for 20 minutes, after which the supernatant was collected and volume measured. The pH of the gastric juice was recorded using a pH meter. Free and total acidity were determined using 0.1 N NaOH for titration, with phenolphthalein and Topfer's reagent as indicators while antioxidant parameters such as superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and malondialdehyde (MDA) in the gastric juice were determined according to standard methods [30, 31, 32, 33].

### Statistical analysis

Results were analyzed using GraphPad Prism 6 (GraphPad Software, Inc. San Diego, California, USA). Experimental data are presented as mean  $\pm$  standard error of mean (SEM) and n represents the number of animals per group. Data comparisons were made using the one way analysis of variance (ANOVA) followed by Dunnett's multiple post hoc test. Values were considered statistically significant at  $p < 0.05$ .

## RESULTS

### Ethanol/Hydrochloric acid-induced ulceration

The type of ulcers seen in this model was the haemorrhagic and long deep type with wide diameter visible from outside the stomach as thick red lines especially in the control group. Treatment with L-glutamine alone significantly ( $p < 0.05$ ) reduced, in a dose-dependent manner, the severity of the ulcers induced by ethanol/hydrochloric acid. Cimetidine (50 mg/kg) caused an attenuation of the ulcer score without any effect on the total number of ulcers (Table 1).

However, treatment with a combination of low dose cimetidine and glutamine significantly ( $p < 0.05$ )

reduced the number and severity of ulcers, when compared with the control.

Misoprostol showed a greater inhibition of ulcer than cimetidine + glutamine though this was not significant.

#### Water immersion stress-induced ulceration

Both low and high doses of cimetidine did not significantly affect the number and severity of ulcers. The lower dose of glutamine (200 mg/kg) showed no protective effect. However the combination of low

dose cimetidine and glutamine significantly ( $p < 0.01$ ) reduced the total number of ulcers and produced an inhibition of 53.1 %.

Omeprazole showed a slightly greater reduction in the number and severity of ulcers than cimetidine + glutamine with an inhibition of 82.17 %

**Table 1: Effect of cimetidine plus L-glutamine combination on ethanol/HCl-induced ulceration in rats**

Treatment groups	Dose (mg/kg)	Mean total ulcer	Mean ulcer score	Ulcer index (UI)	Inhibition (%)
Control	-	6.40 ± 1.17	8.00 ± 0.63	24.40 ± 1.51	-
Cimetidine	10	6.40 ± 2.50	3.40 ± 1.86	17.80 ± 4.32 <sup>a</sup>	27.04
	50	4.60 ± 1.44	1.80 ± 0.80*	16.40 ± 2.20* <sup>a</sup>	32.79
Glutamine	200	0.80 ± 0.37*	1.00 ± 0.45*	7.80 ± 0.80**	68.03
	1000	0.60 ± 0.40*	0.40 ± 0.24*	5.00 ± 0.63**	79.51
Cimetidine + glutamine	10 +200	3.40 ± 2.09	1.40 ± 1.17*	8.80 ± 3.01**	63.93
Misoprostol	0.05	1.80 ± 1.36*	0.40 ± 0.24*	6.20 ± 1.56**	74.59

Values are Mean ± SEM. \* $p < 0.05$ , \*\* $p < 0.01$ , significantly different from control; <sup>a</sup> $p < 0.05$ , compared with Cimetidine+Glutamine; (One way ANOVA; Dunnett's post hoc test). n = 5 animals.

**Table 2: Effect of L-glutamine and cimetidine combination on water immersion stress-induced ulceration in rats**

Treatment groups	Dose (mg/kg)	Mean total ulcer	Mean ulcer score	Ulcer index (UI)	Inhibition (%)
Control	-	14.40 ± 3.54	1.20 ± 0.20	25.80 ± 3.55	-
Cimetidine	10	11.00 ± 1.97	1.10 ± 0.10	22.10 ± 1.90 <sup>a</sup>	14.34
	50	11.40 ± 4.45	0.80 ± 0.20	20.20 ± 4.58 <sup>a</sup>	21.71
Glutamine	200	24.00 ± 5.72	1.00 ± 0.00	35.00 ± 5.72	-
	1000	6.60 ± 2.42*	0.80 ± 0.37	14.90 ± 2.79*	42.25
Cimetidine + glutamine	10 +200	3.20 ± 1.24**	0.90 ± 0.24	12.10 ± 1.44**	53.10
Omeprazole	20	2.00 ± 1.22**	0.20 ± 0.12	4.60 ± 0.37**	82.17

Values are Mean ± SEM. \* $p < 0.05$ , \*\* $p < 0.01$ , significantly different from control; <sup>a</sup> $p < 0.05$ , compared with Cimetidine+Glutamine; (One way ANOVA; Dunnett's post hoc test). n = 5 animals.

**Cysteamine-induced duodenal ulcer**

In the control groups, administration of cysteamine after 24 hours resulted in the production of frank lesions in the proximal segment of the duodenum. Low and high doses of cimetidine and L-glutamine did not protect the duodenum from cysteamine insult (Table 3). However, pre-treatment with cimetidine and

L-glutamine combination significantly inhibited the duodenal ulcers compared to control, although this effect was less than that produced by ranitidine (50 mg/kg). The combination also produced a significant effect ( $p<0.05$ ) on cysteamine-induced gastric ulceration.

**Table 3: Effect of glutamine and cimetidine combination on cysteamine-induced duodenal and gastric ulceration in rats**

Treatment	Dose (mg/kg)	Duodenum		Stomach	
		Ulcer index (UI)	Inhibition (%)	Ulcer index (UI)	Inhibition (%)
Control	-	2.80 ± 0.20	-	11.20 ± 3.20	-
Cimetidine	10	3.00 ± 0.00	-7.14	11.60 ± 0.98	-3.57
	50	2.30 ± 0.60	17.85	3.40 ± 1.40*	69.64
Glutamine	200	2.68 ± 0.15	4.29	6.80 ± 1.71	39.29
	1000	2.08 ± 0.52	25.71	6.40 ± 1.64*	42.86
Cimetidine + Glutamine	10 + 200	1.33 ± 0.54*	52.50	6.00 ± 1.38*	46.43
Ranitidine	50	0.72 ± 0.44*	74.29	3.20 ± 1.20*	71.43

Values are Mean ± SEM. \* $p<0.05$ , significantly different from control; (One way ANOVA; Dunnett's post hoc test). n = 5 animals.

**Table 4: Effect of L-glutamine plus cimetidine on chronic ethanol-induced ulceration in rats**

TREATMENT	Dose (mg/kg/day)	Ulcer index (UI)	Ulcer inhibition (%)	GSH (U/mg pro)	SOD (U/mg pro)	CAT (U/mg pro)	MDA (U/mg pro)
CONTROL	-	7.12±0.97	-	5.37±5.25	7.52±3.22	52.19±12.06	0.99±0.20
Cimetidine	10	0.40±0.20*	94.38	1.61±0.43	7.55±3.01 <sup>a</sup>	38.16±14.23 <sup>a</sup>	0.40±0.20
Cimetidine	50	0.00±0.00**	100.00	2.99±0.78	7.10±0.17 <sup>a</sup>	42.53±8.69 <sup>a</sup>	0.44±0.17
L-glutamine	200	0.22±0.22*	96.91	7.75±1.53	9.02±2.38	56.43±11.26 <sup>a</sup>	0.49±0.20
L-glutamine + Cimetidine (P)	200 +10	3.00±0.76*	51.49	8.72±2.76	18.96±6.03*	106.99±16.77*	0.29±0.13
L-glutamine + Cimetidine (T)	200 +10	0.00±0.00**	100.00	1.99±0.69	6.19±1.70 <sup>a</sup>	38.11±8.33 <sup>a</sup>	0.32±0.05
Ranitidine	50	0.44±0.21*	93.82	1.03±0.30	8.22±2.86 <sup>a</sup>	40.24±14.10 <sup>a</sup>	0.28±0.08

Values are Mean ± SEM. \* $p<0.05$ , \*\* $p<0.01$ , significantly different from control; <sup>a</sup> $p<0.05$ , compared with Cimetidine + Glutamine (P) (One way ANOVA; Dunnett's post hoc test). n = 6 animals. **GSH** = Glutathione; **SOD** = Superoxide dismutase; **CAT** = Catalase; **MDA** = Malondialdehyde, **L-glutamine + Cimetidine (T)** = Therapeutic glutamine-cimetidine dosing; **L-glutamine + Cimetidine (P)** = Prophylactic glutamine-cimetidine dosing

**Ethanol-induced chronic gastric ulcer**

All the treatments significantly ( $p < 0.01$ ) reduced the severity of the ulcers induced by chronic ethanol administration. However, only cimetidine (50 mg/kg/day) and cimetidine plus L-glutamine given therapeutically produced 100 % inhibition of ulcers (Table 4). Treatment with low dose cimetidine plus L-glutamine caused significant ( $p < 0.05$ ) increases in SOD and CAT compared to the control group and other treatment groups. All other parameters were not significantly altered.

**Effect of L-glutamine/cimetidine on sperm parameters and testosterone levels**

No significant changes were observed in the sperm parameters and testosterone with all the treatment protocols compared to control. L-glutamine (200 mg/kg/day) given alone caused appreciable increases in both sperm parameters and testosterone levels (Table 5). Compared to cimetidine alone, both treatment schedules (prophylactic and therapeutic) did not significantly alter the sperm count or motility and testosterone levels.

**Table 5: Effect of L-glutamine and cimetidine combination on sperm parameters and testosterone level in rats**

TREATMENT	Dose (mg/kg)	Sperm motility (%)	Sperm count ( $10^7$ )	Testosterone (ng/dl)
CONTROL	-	85.80±2.37	7.65±0.13	2.68±0.75
Cimetidine	10	80.20±1.11	7.18±0.16	2.24±0.57
Cimetidine	50	74.60±5.18	6.35±0.39	2.24±0.60
L-Glutamine	200	91.60±1.21	8.23±0.26	2.78±0.34
L-Glutamine + Cimetidine (P)	200+10	68.80±1.36	7.20±0.18	3.36±0.71
L-Glutamine + Cimetidine (T)	200+10	85.00±2.84	7.05±0.32	2.06±0.37
Ranitidine	50	80.40±2.16	5.80±0.38	2.08±0.34

Values are Mean ± SEM. (One way ANOVA; Dunnett's post hoc test). n = 5 animals.

L-glutamine + Cimetidine (T) = Therapeutic glutamine-cimetidine dosing;

L-glutamine + Cimetidine (P) = Prophylactic glutamine-cimetidine dosing

**Table 6: Effect of L-glutamine and cimetidine combination on indomethacin plus pylorus ligation-induced ulceration in rats**

Treatment	Dose (mg/kg/day)	Mean total ulcer	Mean ulcer score	Ulcer index	Inhibition (%)
CONTROL	-	7.00±1.28	13.50±1.28*	31.17±2.23	-
Cimetidine	50	5.17±1.46	8.00±1.51* <sup>a</sup>	21.33±3.05*	31.57
Ranitidine	50	3.20±1.02**	7.60±1.88*	20.80±2.65*	33.27
Omeprazole	20	4.60±0.81*	9.00±1.66* <sup>a</sup>	23.20±2.53*	25.57
L-glutamine	200	3.67±1.24**	6.33±2.22**	18.16±2.96**	41.74
L-glutamine + Cimetidine	200+10	3.00±1.00**	5.80±2.62**	17.13±3.12**	45.04

Values are Mean ± SEM. \* $p < 0.05$ , \*\* $p < 0.01$ , significantly different from control; <sup>a</sup> $p < 0.05$ , compared with L-glutamine + Cimetidine (ANOVA; Dunnett's post hoc test). n = 5-6 animals.

### Indomethacin plus pylorus ligation-induced ulcer

The combination of L-glutamine and cimetidine significantly reduced both the number and severity of the ulcer induced with indomethacin plus pyloric ligation, compared to control. Its effect was also significantly different from that of cimetidine alone (Table 6).

None of the treatments caused any significant changes in the volume, pH or acidity (free or total) of the gastric juice (Fig. 7).

Table 8 shows the effect of various treatments on oxidant and antioxidant enzymes measured in gastric juice of rats in which ulcer was induced with indomethacin plus pyloric ligation. With the exception of omeprazole, none of the treatments produced any significant changes in the gastric juice enzymes.

**Table 7: Effect of L-glutamine/cimetidine combination on gastric juice parameters in indomethacin plus pylorus ligation-induced ulceration in rats**

Treatment	Dose (mg/kg/day)	Vol. of gastric juice (ml)	pH	Free Acidity (mEq/L)	Total acidity (mEq/L)
CONTROL	-	1.34±0.40	5.48±0.51	14.33±2.08	44.83±2.86
Cimetidine	50	1.66±0.17	5.59±0.81	17.20±2.35	44.48±4.55
Ranitidine	50	2.08±0.31	6.08±0.73	18.40±2.25	52.50±4.15
Omeprazole	20	3.80±1.07	6.50±0.45	22.00±1.41	50.20±2.31
L-glutamine	200	1.68±0.25	6.00±0.44	20.50±2.08	45.33±2.92
L-glutamine + Cimetidine	200 + 10	2.97±0.76	6.85±0.14	25.20±3.65	43.40±9.98

Values are Mean ± SEM. (ANOVA; Dunnett's post hoc test). n = 5-6 animals.

**Table 8: Effect of L-glutamine and cimetidine combination on antioxidant parameters in indomethacin + pylorus ligation-induced ulceration in rats**

TREATMENT	Dose (mg/kg)	GSH (U/mg prot)	SOD (U/mg prot)	CAT (U/mg prot)	MDA (U/mg prot)
CONTROL	-	0.26±0.08	2.42±0.30	8.17±1.09	0.07±0.00
Cimetidine	50	0.62±0.18	2.90±0.30	12.38±1.14	0.06±0.02
L-glutamine	200	0.59±0.10	2.17±0.21	9.46±0.95	0.03±0.01
L-glutamine + Cimetidine	+10	0.49±0.05	0.99±0.09	4.62±0.37	0.03±0.00
Ranitidine	50	0.41±0.06	2.61±0.37	11.17±1.98	0.02±0.00*
Omeprazole	20	0.78±0.20*	3.81±0.69	15.11±3.00*	0.02±0.03*

Values are Mean ± SEM. \*p<0.05, significantly different from control (ANOVA; Dunnett's post hoc test). n = 5-6 animals. **GSH** = Glutathione; **SOD** = Superoxide dismutase; **CAT** = Catalase; **MDA** = Malondialdehyde

## DISCUSSION

This study was carried out to evaluate the gastroprotective efficacy of low dose cimetidine and L-glutamine utilizing the ethanol/hydrochloric acid-, water immersion stress-, cysteamine-, chronic ethanol- and indomethacin/pylorus ligation-induced models of ulcer in rats.

The results of the study show that a combination of low dose cimetidine with L-glutamine exerted

protective effects against acute and chronically induced gastric and duodenal mucosal damage.

The anti-ulcer effect of cimetidine and L-glutamine combination was tested against gastric lesions induced by ethanol/HCl, the experimental model related to lesion pathogenesis with production of reactive oxygen species.

Hydrochloric acid is known to cause severe damage to the gastric mucosa. Oral administration of ethanol causes necrotic lesions of the gastric mucosa in various ways, including damage to mucosal cells and mucosal blood flow, back diffusion of acid and

disruption of the mucus-bicarbonate barrier [36]. Hence acidification of ethanol by addition of HCl has a synergistic effect resulting in increased levels of stomach acidity, lipid peroxidation and a massive corrosive effect on the gastric mucosa leading to ulceration [37].

L-glutamine alone at varying doses was effective in preventing development of ethanol/HCl induced gastric ulcer unlike cimetidine alone which showed a slight gastroprotective effect. Cimetidine + L-glutamine was effective in preventing the development of ethanol/HCl-induced ulcer indicating that L-glutamine enhances the gastroprotective efficacy of low dose cimetidine hence a synergistic effect exists between the two drugs.

Water immersion stress-induced ulcer is associated with increased gastric acid secretion and a decrease in pH which causes digestion of mucus membrane and formation of a characteristic lesion on the gastric mucosa. Histamine is believed to play an essential role in the pathogenesis of stress induced ulcer since it is a potent stimulator of gastric acid secretion [38].

Oxygen-derived free radicals, such as the superoxide anion, hydrogen peroxide and hydroxyl radical, play an important role in the pathogenesis of the injury of various tissues, including the digestive system [39, 40].

Cimetidine and glutamine alone, respectively, had mild gastro-protective effect which was dose dependent although the lower dose of glutamine did not show any effect, either on the number or severity (score) of ulcers. The combination of cimetidine and glutamine resulted in much greater gastroprotective efficacy compared to cimetidine or L-glutamine alone. This further confirms the synergistic effect between L-glutamine and cimetidine, which may serve to reduce some of the side effects associated with cimetidine use at high doses and/or chronic use.

Cysteamine-induced duodenal ulcers are considered to be due to a long-lasting hypersecretion of gastric acid, deterioration of mucosal resistance, increased serum gastrin concentration as well as promotion of gastric emptying [41, 42, 43]. Results of the present study suggest that the severity of duodenal ulcers was, significantly, reduced relative to control after oral pretreatment with combination of low doses of L-glutamine and cimetidine. Interestingly, both low doses of cimetidine and L-glutamine given separately did not protect the stomach from the insult by

cysteamine but conferred over 50 % protection from duodenal ulcers when given together. Cimetidine has been found to be less effective in rats against cysteamine-induced duodenal ulceration [44]. This significant effect by the combination is a further proof of their synergistic effect on ulcer.

Using the chronic ulcer model, administration of ethanol for 7 days induced mucosal lesions in control rats, reflected as haemorrhage, erosions and frank ulcer. Lesions resulting from chronic administration of ethanol to rats have been attributed to mucus depletion, and constriction of the gastric mucosal blood vessels, producing congestion, inflammation and tissue injury [45]. The reduction of gastric mucosal blood flow can result in haemorrhage and necrosis in damaged tissue [46, 47]. Ethanol also triggers imbalances in cellular antioxidant processes [48].

The combination of L-glutamine and cimetidine given 8 days after induction of chronic ethanol ulcer (therapeutic dosing) produced a complete inhibition of ulcer without any effect on the antioxidants. However, prophylactic administration caused a mild inhibition of ulcer (51.5 %) with significant elevations in superoxide dismutase (SOD) and catalase (CAT), relative to control. The reduced glutathione (GSH) level was increased and the MDA (a lipid peroxidation product) was reduced but not significantly. These effects described demonstrate the broad antioxidant properties of this combination and its efficacy in preventing free-radical induced damage in the stomach tissues. The preventive antioxidants, SOD and catalase, are the first line of defense against reactive oxygen species while GSH is a major low molecular weight scavenger of free radicals in the cytoplasm and an important inhibitor of free radical-mediated lipid peroxidation [49].

The effectiveness of the combination of L-glutamine and cimetidine in preventing gastric ulcer via anti-secretory activity was tested against indomethacin/pylorus ligation-induced ulceration.

Indomethacin causes disturbances in gastric secretion, damage to gastric mucosa, alteration in permeability, gastric mucus depletion and increase in the pepsin and protein content [50]. Indomethacin is known to induce the reactive oxygen metabolites in animal models, which may contribute to mucosal injury [51]. These free radicals also damage the cellular antioxidant enzymes such as CAT, SOD and others, acting as the first line of cellular defense

against oxidative injury and leading to aggravated tissue damage during stomach ulceration [52].

Pylorus ligation causes accumulation of gastric acid and pepsin in the stomach resulting in autodigestion of gastric mucosa and breakdown of gastric mucosal barrier with consequent development of gastric ulcers [53, 54]. Reactive oxygen species are also involved in the pathogenesis of pylorus ligation ulcer [49].

The combination of L-glutamine and cimetidine caused greater reduction in the number and severity of ulcers than either ranitidine or omeprazole. The combination also increased the pH without affecting the volume and output of the gastric juice. This suggests that the combination may not have anti-secretory properties as gastric acid is an important factor for the genesis of ulceration in pylorus-ligated rats.

The finding that the combination did not exert any significant effect on the gastric juice parameters in contrast to the tissue antioxidants which were significantly altered, confirmed our belief that the healing or prevention of gastric and duodenal ulcers may not be due to its effect on gastric juice secretion.

Cimetidine is a known reproductive toxicant which significantly reduces weight of accessory sex organs [55] causing gynaecomastia in males [11] and galactorrhea in females [56, 57]. These side effects are believed to result from raised levels of prolactin which directly lead to a decreased level of testosterone and a resultant decrease in sperm count. Although not statistically significant, the combination of L-glutamine with cimetidine showed slight improvements in sperm parameters (count and motility) as well as in blood testosterone levels compared with cimetidine (50 mg/kg) given alone.

The combination of low dose cimetidine and L-glutamine showed significant effects in most of the models of ulcer used which is an indication of its efficacy in treatment of peptic ulcer. The combination also showed a greater gastroprotective efficacy compared to either cimetidine or glutamine alone, which points to a synergistic action between the two drugs. Since reducing the dose of a drug tends to reduce its side effects, the results therefore suggest that low dose cimetidine and glutamine combination could hold promise as a better and safer therapy than ranitidine and the proton pump inhibitors in the management of peptic ulcer disease. Ranitidine use is associated with adverse effects such as thrombocytopenia, decreased libido, anorgasmia and

impotence, while long-term proton pump inhibitor (e.g. omeprazole) therapy has been implicated in hypergastrinaemia and gastric cancer [58]. However, the lack of significant effect of the combination on some of the known side effects of long term use of cimetidine at therapeutic doses may be a function of the doses used and the duration of treatment. Further work would, therefore, be necessary in this direction.

## COMPETING INTERESTS

The authors declare no competing interests.

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