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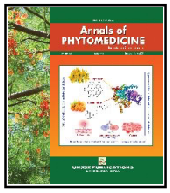

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Racecadotril as a novel therapeutic approach for indomethacin plus pyloric ligation induced gastric ulcer management in rats: An *in vivo* study

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Abstract

Gastric ulcers, often induced by NSAIDs and gastric hypersecretion, remain a prevalent clinical concern. This study aimed to evaluate the acid-inhibitory and gastroprotective effects of Racecadotril (Race) in indomethacin plus pyloric ligation-induced gastric ulcer in Sprague Dawley rats. Animals were divided into nine groups and received either CMC, indomethacin, Racecadotril (100 or 200 mg/kg), or ranitidine for the respective treatment durations. Ulcer index, gastric pH, gastric juice volume, mucus content, oxidative stress markers (TBARS), and antioxidant enzymes (GSH, CAT, SOD) were assessed. Results showed that the indomethacin plus pyloric ligation group exhibited a high ulcer index (28.33 ± 0.05), increased gastric volume (5.3 ± 0.44 ml), and reduced mucus (99.28 ± 1.64 µg/g). In contrast, Racecadotril at 200 mg/kg significantly reduced ulcer index (15.88 ± 0.19), increased pH (3.5 ± 0.12), lowered gastric juice volume (1.70 ± 0.27 ml), and restored mucus (173.94 ± 0.94 µg/g). TBARS levels decreased while GSH, CAT, and SOD levels improved markedly. These findings suggest that Racecadotril exhibits significant dose-dependent gastro protective effects through acid suppression, mucus preservation, and antioxidant enhancement, making it a promising alternative for gastric ulcer management.

1. Introduction

Gastric ulcers, a prevalent form of peptic ulcer disease, pose significant clinical challenges due to their complex pathophysiology and the potential for severe complications such as bleeding and perforation (Pujari *et al*., 2024). The development of gastric ulcers is predominantly due to an imbalance between aggressive factors like hydrochloric acid and pepsin, and the defensive mechanisms of the gastric mucosa, including mucus and bicarbonate secretion (Khan *et al*., 2023). This imbalance is exacerbated by factors such as stress, smoking, alcohol consumption, and notably, the use of non-steroidal anti-inflammatory drugs (NSAIDs) like aspirin, diclofenac. These NSAIDs inhibit cyclooxygenase (COX) enzymes, leading to reduced prostaglandin synthesis, which is crucial for maintaining gastric mucosal integrity (Bjarnason *et al*., 2018). In research, animal models using agents like indomethacin combined with techniques such as pyloric ligation (PL) are often employed to simulate these conditions and evaluate potential treatments for their efficacy in preventing or ameliorating experimental ulcer formation (Khatoon *et al*., 2024).

Racecadotril, known primarily for its antisecretory effects in the treatment of acute diarrhea, acts as a pro drug that is metabolized to thiorphan, an enkephalinase inhibitor. By blocking this enzyme, Racecadotril enhances the levels of endogenous opioids that decrease intestinal hypersecretion (Szymaszkiewicz *et al*., 2019). Despite its established use in managing diarrhea, the effects of Racecadotril on gastric secretion and its potential protective effects against gastric ulcers remain underexplored. The drug's mechanism suggests a possible dual action not only in reducing intestinal fluid secretion but also potentially modulating gastric acid secretion. This research aims to explore these anti-secretory and protective properties in a model of gastric ulcer induced by indomethacin and pyloric ligation in Sprague Dawley rats, providing a novel perspective on the drug's utility in gastrointestinal pharmacotherapy.

This study is structured to systematically assess the effects of Racecadotril in the context of indomethacin induced gastric damage, which is highly relevant given the widespread use of indomethacin and the associated risk of gastric ulceration. The methodology involves administering Racecadotril prior to the induction of ulcers to determine its prophylactic efficacy. By employing a well-established animal model that combines drug-induced and acid induced factors, the research will offer comprehensive insights into the drug's impact on both gastric acid secretion and mucosal defense mechanisms. Furthermore, this study will provide a detailed analysis of Racecadotril's gastroprotective effects. Given the limitations and side effects associated with current anti-ulcer medications, such as proton pump inhibitors and H_2 receptor antagonists, Racecadotril could represent a valuable alternative with a potentially safer profile and a novel mechanism of action.

2. Materials and Methods

2.1 Drugs and chemicals

Ranitidine (GSK, India), Indomethacin (Jagsonpal, India), Racecadotril (Dr Reddy's Laboratories Ltd), 5,5-dithiobis-2-nitro benzoic acid

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(DTNB, Sigma-Aldrich, USA), Alcian blue, Pyrogallol, Folin Ciocalteau phenol reagent, Diethyl ether, Methanol, Sucrose were from SD-Fine chemicals. Chemicals and buffers of the analytical grade were used.

2.2 Animals

Adult male Sprague Dawley Rats body weight (150-200 g) procured from Central Drug Research Institute (CDRI), Lucknow. The animals were kept in polypropylene cages (5 in each cage) under standard laboratory conditions (12 h light and 12 h dark at day and night cycle) and had a free access to appropriate diet and tap water ad libitum. The animal house temperature was maintained at 23 ± 2°C and relative humidity also maintained at (50 ± 15%). The gastric ulcer was induced by pyloric ligation and indomethacin plus pyloric ligation method. Ethical clearance was obtained from Institutional Animal Ethics Committee (Approval No. IU/IAEC/22/08).

2.3 Experimental design

The animals were divided into 9 groups, each containing 5 rats (n=5, number of rats per group). Group I, serving as the normal control (NC) for ulcer index, antioxidants and mucus, received 1% carboxyl methyl cellulose (CMC; 1 ml/kg/day, orally) for 7 days. Group II, designated as disease control indomethacin plus pyloric, underwent pretreatment with CMC for 7 days, followed by administration of indomethacin (20 mg/kg/day, orally) 6 h prior to pylorus ligation under ketamine anesthesia (40 mg/kg, intraperitoneally) after a 24 h fast. Group III, identified as pyloric ligation, also received 7 days of 1% CMC, followed by pylorus ligation under ketamine anesthesia on the 8th day. Group IV (Racecadotril at 100 mg/kg/p.o./day plus pyloric ligation) and Group VII (Racecadotril at 200 mg/kg/day plus pyloric ligation) were treated with Racecadotril at doses of 100 mg/kg/day and 200 mg/kg/p.o./day, respectively, administered orally for 7 days, with pylorus ligation performed on the 8th day under ketamine anesthesia. Similarly, Group V (Race at 100 mg + Indo + Pyloric) and Group VI (Race at 200 mg + Indo + Pyloric) received Racecadotril for 7 days at their respective dosages, followed by indomethacin administration on the eighth day and subsequent pylorus ligation 6 h later, all under ketamine anesthesia after fasting for 24 h. Group VIII (Ranitidine (50 mg/kg/day, orally) + Indo + Pyloric) and Group IX (Ranitidine (50 mg/kg/day, orally) + Pyloric) were pre-treated with ranitidine (50 mg/kg/day, orally) for 7 days. In Group VIII, indomethacin was administered on the 8th day, 6 h prior to pylorus ligation, similar to Group II. Group IX underwent only pylorus ligation after the ranitidine pretreatment period. All treatments involving pylorus ligation and indomethacin administration were performed under similar fasting conditions and anesthesia protocols to ensure consistency across the experimental groups (Table 1).

Table 1: Treatment protocol

Groups(n=5)	Treatments
Group I NC	1% Carboxy methyl cellulose (CMC; 1 ml/kg/day, p.o.) daministration for 7 days.
Group II Indo + PL	Pretreatment with 1% CMC (1 ml/kg/day, p.o.) for 7 days plus indomethacin (20 mg/kg/day, p.o.) administration on 8th day before 6 h of pylorus ligation under ketamine (40 mg/kg, i.p.) anesthesia
Group III PL	1% CMC (1 ml/kg/day, p.o.) for 7 days plus pylorus ligation on 8th day under ketamine (40 mg/kg i.p.) anesthesia.
Group IV Race (100 mg) + PL	Racecadotril (100 mg/kg/day, p.o.) for 7 days plus pylorus ligation on 8th day under ketamine (40 mg/kg i.p.) anesthesia.
Group V Race (100 mg) + Indo + PL	Pretreatment with Racecadotril (100 mg/kg/day, p.o.) for 7 days plus indomethacin (20 mg/kg/day, p.o.) administration on 8th day before 6 h of Pylorus ligation under ketamine (40 mg/kg ip) anesthesia on 24 h fasted rats.
Group VI Race (200 mg) + Indo + PL	Pretreatment with Racecadotril (200 mg/kg/day, p.o.) for 7 days plus indomethacin (20 mg/kg/day, p.o.) administration on 8th day before 6 h. of pylorus ligation under ketamine (40 mg/kg i.p.) anesthesia.
Group VII Race (200 mg) + PL	Racecadotril (200 mg/kg/day, p.o.) for 7 days plus pylorus ligation on 8th day under ketamine (40 mg/kg i.p.) anesthesia.
Group VIII Ranitidine (50 mg/kg) + Indo + PL	Pretreatment with ranitidine (50 mg/kg/day, p.o.) for 7 days plus indomethacin (20 mg/kg/day, p.o.) administration on 8th day before 6 h of pylorus ligation under ketamine anesthesia (40 mg/kg i.p.).
Group IX Ranitidine (50 mg/kg) + PL	Pretreatment with ranitidine (50 mg/kg/day, p.o.) for 7 days plus pylorus ligation on 8th day under ketamine (40 mg/kg i.p.) anesthesia.

Once anesthetized, a small midline incision was made below the xiphoid process to open the abdomen, and the pyloric portion of the stomach was gently lifted and ligated, ensuring no traction on the pylorus or damage to its blood supply. The stomach was carefully repositioned, and the abdominal wall was closed using interrupted sutures. During the postoperative period, the animals were deprived of both food and water, and gastric juice was allowed to accumulate for 4 h. After this period, the rats were sacrificed by decapitation under anaesthesia, and their stomachs were removed after clamping the oesophagus. The gastric contents were collected through the oesophagus into a test tube. The stomachs of both models were exposed along the greater curvature, cleaned with distilled water, and examined for lesions. The gastric juice was collected in a centrifuge tube, and after centrifugation, the volume, pH, and total acidity of the gastric juice were measured.

2.4 Evaluation parameters

The evaluation of the treatment outcomes were based on following parameters:

2.4.1 Estimation of ulcer index

The ulcer index was used to assess the severity of gastric lesions in the stomach. This index was calculated by scoring the number and size of ulcers observed in the stomach, which provides a quantitative measure of gastric damage (Khan *et al*., 2024).

2.4.2 pH of gastric juice

The pH of gastric juice was measured to evaluate the acidity of the stomach contents, which plays a crucial role in gastric mucosal injury. A pH meter was used to determine the pH of the collected gastric juice, providing insight into the gastric environment under different treatment conditions (El-Ashmawy *et al*., 2016).

2.4.3 Volume of gastric juice

The volume of gastric juice was also recorded, as it is an important indicator of gastric secretion. The volume was measured after the accumulation period to assess the secretion dynamics and its potential alteration due to the treatment protocol (Tan *et al*., 2023)

2.4.4 Gastric wall mucus (barrier mucus) determination

Gastric wall mucus was determined by using the method of (Tan *et al*., 2023; Khan *et al*., 2024).

2.4.5 Biochemical estimations

Biochemical estimations were carried out to assess oxidative stress and the biochemical changes in the gastric mucosa. The levels of superoxide dismutase (SOD) and catalase (CAT) were measured to evaluate the antioxidant status and the ability of the gastric tissue to neutralize reactive oxygen species. The concentration of thiobarbituric acid reactive substances (TBARS) was measured to determine the extent of lipid peroxidation and oxidative damage in the gastric tissues. These biochemical markers provided a deeper understanding of the cellular and molecular mechanisms underlying the effects of the treatment (Khan *et al*., 2024).

2.4.6 Macroscopic evaluation

Macroscopic evaluation of the stomach was performed to visually examine any visible lesions, inflammation, or other abnormalities in the gastric mucosa. This evaluation provided a direct observation of the physical condition of the stomach, helping to correlate the severity of damage with the treatment groups (Khan *et al*., 2024; Shariq *et al*., 2023).

2.5 Statistical analysis

The analysis was expressed as mean ± SD. Statistical analysis was performed using one way ANOVA, followed by Dunnett test using GraphPad Prism Software.

3. Results

3.1 Effect on ulcer index

Table 2 presents the mean and standard deviation (SD) of the ulcer index across different experimental groups. The indomethacin plus pyloric ligation (Indo + PL) group exhibited a significantly elevated ulcer index (Mean = 28.33 ± 0.05), confirming the ulcerogenic potential of this model. In contrast, NC group showed no ulceration, validating the baseline physiological condition.

Among the treatment groups, Racecadotril in combination with indomethacin and pyloric ligation at 200 mg/kg markedly reduced the ulcer index (Mean = 18.37 ± 0.20), indicating a protective effect. Notably, Indo + PL alone resulted in the highest ulcer index (Mean = 28.34 ± 0.05), while the Racecadotril 100 mg/kg group showed the least protective effect (Mean = 29.33 ± 0.17), suggesting a dose-dependent response.

These findings imply that Racecadotril, particularly at a higher dose, confers significant gastro protection against indomethacin-induced ulceration, supporting its potential role in anti-ulcer therapy.

3.2 Effect on pH of gastric juice

Table 2 summarizes the mean ± standard deviation (SD) of gastric pH values across all experimental groups. The group treated with indomethacin and subjected to pyloric ligation (Indo + PL) showed a significantly lower gastric pH of 1.7 ± 0.33 when compared to the CMC + PL group ($p< 0.01$). This marked reduction in pH reflects a hyper acidic state induced by the combined ulcerogenic insult, validating the reliability of this experimental ulcer model.

Notably, treatment with Racecadotril and ranitidine in ulcer-induced models led to a substantial increase in gastric pH, suggesting acid-inhibitory properties. For instance, the Race 200 mg/kg + Indo + PL group showed a pH of 3.5 ± 0.12, while the Ranitidine + Pyloric group reached 3.9 ± 0.09. These elevations in pH were statistically significant when compared with the Indo + PL group ($p< 0.01$ to $p< 0.001$). These findings demonstrate the potential of both drugs to mitigate gastric acidity under ulcer-inducing conditions.

Furthermore, when groups were compared to the PL group, a significant increase in pH was observed in several Racecadotril-treated groups, including Race (100 mg/kg) + Pyloric ligation and Race (200 mg/kg + Pyloric), with pH values of 2.5 ± 0.18 and 3.1 ± 0.12, respectively. These differences, marked with a '*', reached statistical significance ($p<0.05$), suggesting that even in the absence of indomethacin, Racecadotril conferred partial protection by elevating gastric pH.

3.3 Effect on gastric juice volume

Table 2 presents the mean ± standard deviation (SD) of gastric juice volume across the experimental groups. The Indo + PL group demonstrated a significantly elevated gastric juice volume (5.3 ± 0.44, $p< 0.001$) when compared to the CMC + PL group, denoted by the symbol '#', confirming the induction of gastric hyper secretion under ulcerative conditions.

Groups receiving Racecadotril and ranitidine showed marked reductions in gastric juice volume. When compared to the Indo + PL group, groups such as Race (200 mg/kg) + Indo + PL, Ranitidine + Indo + PL, and Race (100 mg/kg) + Indo + PL exhibited significantly lower volumes, with adjusted p-values ranging from 0.00003 to 0.013, indicated by the '#' symbol. These reductions suggest a strong inhibitory effect on gastric secretion in the presence of ulcer-inducing stimuli. Similarly, comparisons with the CMC + PL group revealed that Race (100 mg/kg) + PL, Race (200 mg/kg) + Pyloric, and Ranitidine + PL significantly decreased gastric juice volume ($p<0.05$ to $p<0.001$), denoted by '*'. This points toward a partial protective effect of these treatments even in the absence of indomethacin.

Table 2: Effect of Racecadotril, indomethacin and ranitidine on ulcer index, pH of gastric luice, gastric volume and gastric wall mucus.

Groups (n=5)	Ulcer index	pH of gastric juice	Gastric juice volume	Gastric wall mucus (µg/g of stomach wt.)
Group I NC	0	0	0	219.2 ± 0.34
Group II Indo + PL	25.66 ± 1.37#	1.7 ± 0.33#	5.3 ± 0.44#	99.28 ± 1.64#
Group III PL	28.33 ± 0.05*	2.2 ± 0.12*	4.24 ± 0.33*	130.48 ± 1.21*
Group IV Race (100 mg/kg) + PL	15.61 ± 0.75*	2.5 ± 0.18*	2.73 ± 0.48*	191.28 ± 1.07*
Group V Race (100 mg/kg) + Indo + PL	17.32 ± 0.16#	2.9 ± 0.18#	3.88 ± 0.13#	171.66 ± 0.20#
Group VI Race (200 mg/kg) + Indo + PL	15.88 ± 0.19#	3.5 ± 0.12#	1.70 ± 0.27#	173.94 ± 0.94#
Group VII Race (200 mg/kg) + PL	14.03 ± 0.20*	3.1 ± 0.12*	3.7 ± 0.23*	184.07 ± 0.91*
Group VIII Ranitidine (50 mg/kg) + Indo + PL	13.35 ± 0.19#	3.7 ± 0.09#	2.42 ± 0.27#	179.1 ± 0.65#
Group IX Ranitidine (50 mg/kg) + PL	12.69 ± 0.37*	3.9 ± 0.09*	1.78 ± 0.19*	196. 56 ± 1.27*

Comparisons are performed using ANOVA, followed by Dunnett's test. Values are expressed as mean ± standard deviation (SD) for each group (n = 5). * indicates a statistically significant difference (p<0.001) when compared to the PL group. # indicates a statistically significant difference (p<0.001) when compared to the Indo + PL group.

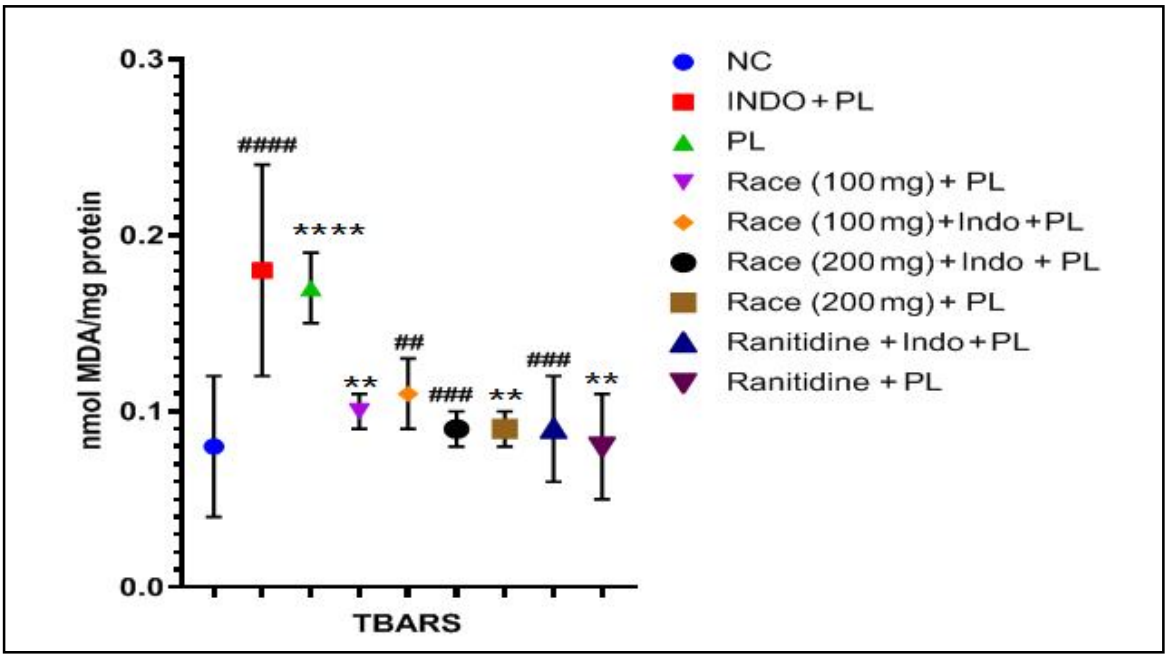

Figure 1 : TBARS estimation in various treatment groups.
All values were expressed as mean ± SD; (n=5). Statistical analysis was done by one-way ANOVA, followed by Dunnett's test, where Race (100 mg) + PL, Race (200 mg) + PL and Ranitidine + PL were compared with PL, Race (100 mg) + Indo + PL, Race (200 mg) + Indo + PL and Ranitidine + Indo + PL were compared with Indo + PL and Indo + PL and PL were compared with NC. ** and ## show significance level at p<0.01, ### shows significance level at p<0.001 and **** and #### show significance level at p<0.0001.

3.4 Effect on gastric wall mucus

Table 2 demonstrates the effects of various treatments on gastric mucus content, expressed as mean ± standard deviation (SD) in µg/g of stomach tissue. The NC group exhibited the highest mucus content (219.2 ± 0.34), representing the intact mucosal defense in healthy rats. In contrast, the Indo + PL group showed a substantial decrease in gastric mucus levels (99.28 ± 1.64), indicating mucosal injury and confirming the efficacy of the ulcer induction model.

Treatment with Racecadotril and ranitidine, both alone and in combination with ulcer-inducing agents, resulted in marked increases in gastric mucus content. Notably, the Ranitidine + PL (IX group) (196.56 ± 1.27) and Race (100 mg/kg) + PL group (191.28 ± 1.07) demonstrated mucus levels approaching that of the NC group, suggesting a potent mucosal protective effect. Groups receiving Racecadotril in combination with Indo + PL (at both 100 mg/kg and 200 mg/kg doses) also showed significant recovery of mucus content compared to the Indo + PL group.

3.5 Effect on malondialdehyde (MDA), glutathione (GSH), catalase (CAT), superoxide dismutase (SOD)

Figures 1-4 displays the biochemical markers of oxidative stress and antioxidant defense-TBARS, GSH, CAT, and SOD-across different treatment groups. The Indo + PL group exhibited significant oxidative damage, characterized by elevated TBARS levels (0.18 ± 0.06) and a pronounced depletion in antioxidant defenses (GSH: 4.96 ± 0.08, CAT: 0.05 ± 0.01, SOD: 0.25 ± 0.02), all highly significant compared to the control group (p<0.001, denoted by '#'). In contrast, treatment groups receiving Racecadotril and Ranitidine, especially in higher doses and in ulcer-induced models, demonstrated substantial biochemical recovery. For instance, Ranitidine + Pyloric and Race (200 mg/kg) + PL groups showed TBARS levels near those of the control group, alongside significantly restored GSH, CAT, and SOD levels (p<0.001, denoted by '*' or '#'). These biochemical improvements were also evident in Race (200 mg/kg) + Indo + PL and Ranitidine + Indo + PL groups, reinforcing their antioxidant and mucosal protective roles. These results confirm that Racecadotril and ranitidine treatments effectively mitigate oxidative stress and enhance antioxidant capacity, thereby playing a critical role in gastric mucosal protection against experimentally induced ulcers.

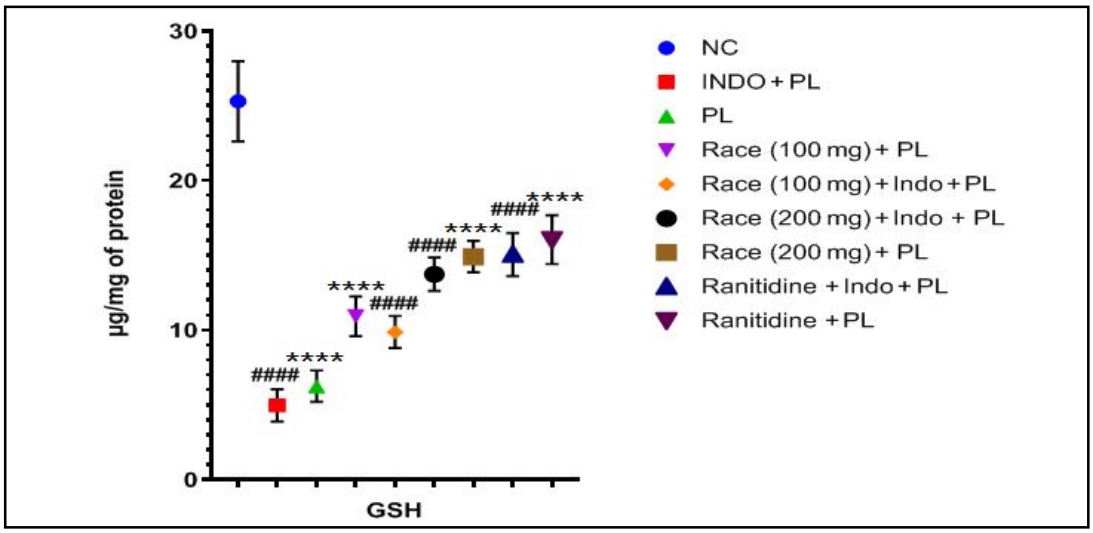

Figure 2 : GSH estimation in various treatment groups.

All values were expressed as mean ± SD; (n=5). Statistical analysis was done by one-way ANOVA, followed by Dunnett's test, where Race (100 mg/kg) + PL, Race (200 mg/kg) + PL and Ranitidine + PL were compared with PL, Race (100 mg/kg) + Indo + PL, Race (200 mg/kg) + Indo + PL and Ranitidine + Indo + PL were compared with Indo + PL and Indo + PL and PL were compared with NC. **** and #### show significance level at p<0.0001.

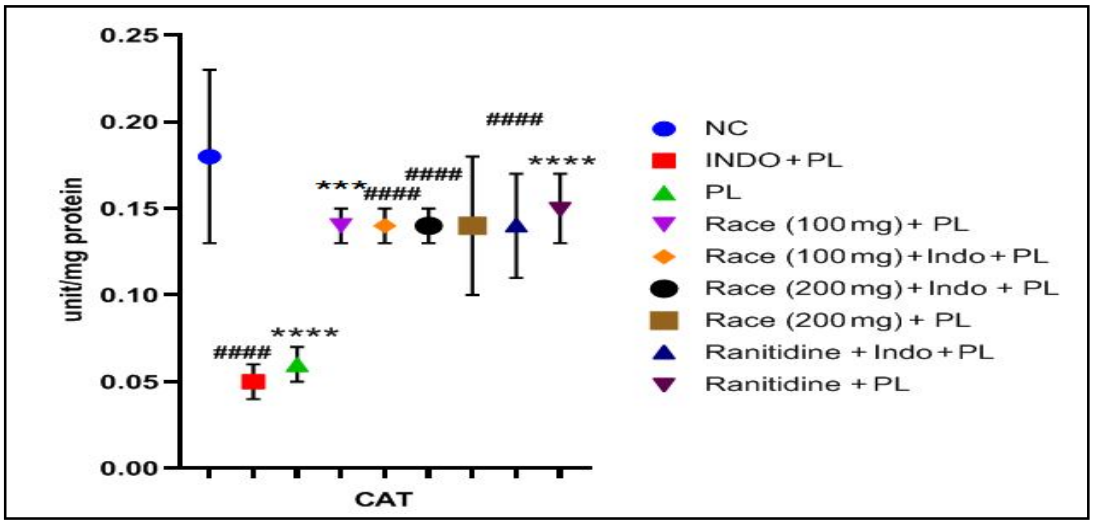

Figure 3 : CAT estimation in various treatment groups.

All values were expressed as mean ± SD; (n=5). Statistical analysis was done by one-way ANOVA, followed by Dunnett's test, where Race (100 mg/kg) + PL, Race (200 mg/kg) + PL and Ranitidine + PL were compared with PL, Race (100 mg/kg) + Indo + PL, Race (200 mg/kg) + Indo + PL and Ranitidine + Indo + PL were compared with Indo + PL and Indo + PL and PL were compared with NC. *** shows significance level at p<0.001 and **** and #### show significance level at p<0.0001.

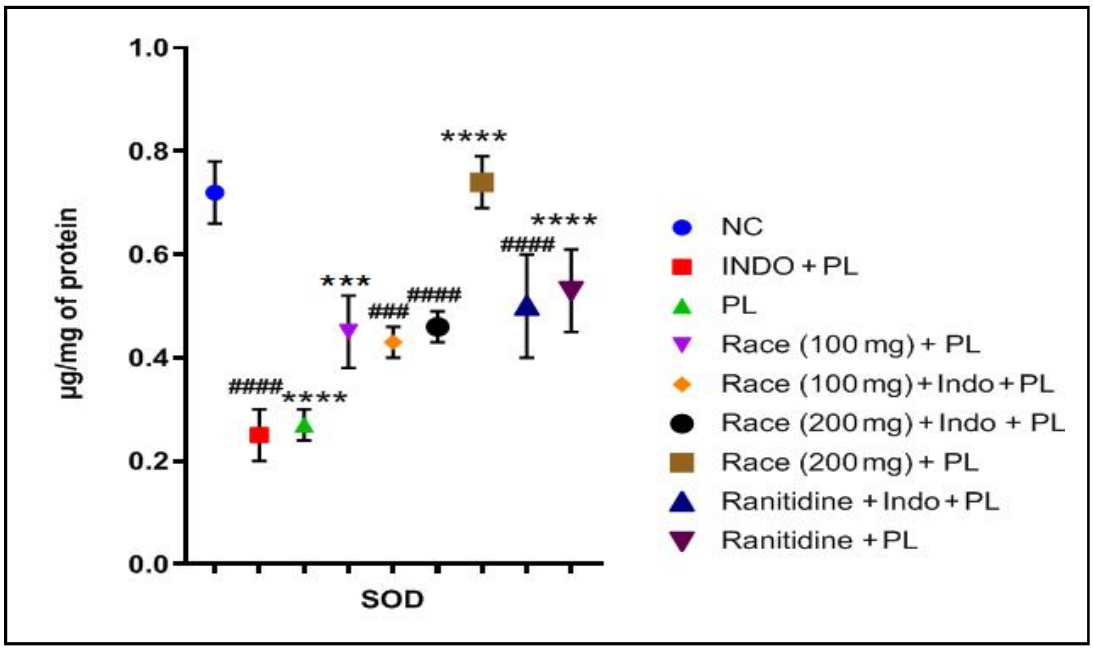

Figure 4 : SOD estimation in various treatment groups.

All values were expressed as mean ± SD; (n=5). Statistical analysis was done by one-way ANOVA, followed by Dunnett's test, where Race (100 mg/kg) + PL, Race (200 mg/kg) + PL and Ranitidine + PL were compared with PL, Race (100 mg/kg) + Indo + PL, Race (200 mg/kg) + Indo + PL and Ranitidine + Indo + PL were compared with Indo + PL and Indo + PL and PL were compared with NC. *** and ### show significance level at $p<0.001$, and **** and #### show significance level at $p<0.0001$.

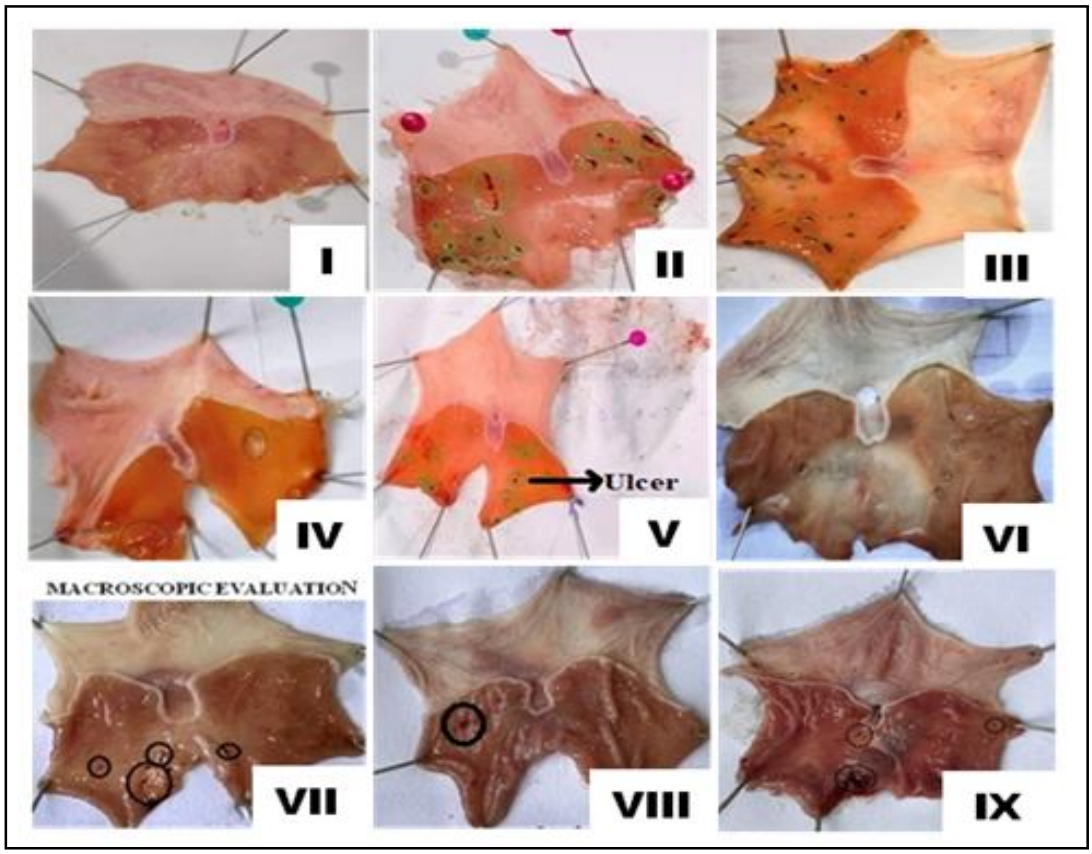

Figure 5: Macroscopic evaluation of the stomach.

3.6 Macroscopic evaluation

The severity of gastric lesions varies significantly, illustrating the influence of both the therapeutic interventions and the model used (Figure 5). Group II, which served as a model for severe gastric ulceration due to the combined stress of NSAIDs (indomethacin) and PL, exhibited extensive ulceration. This group underscores the aggressive nature of NSAIDs when coupled with pyloric ligation, which collectively exacerbates gastric mucosal damage due to reduced prostaglandin synthesis and increased gastric acid exposure. In contrast, Group V, treated with a lower dose of Racecadotril (100 mg/kg) alongside the ulcer-inducing agents, showed a moderate reduction in lesion severity. This suggests that Racecadotril can mitigate some of the detrimental effects induced by NSAIDs and pyloric ligation, likely through its anti-secretory properties, although its protective mechanism might also involve modulation of local neuropeptides and inflammatory mediators. Further improvements were noted in Group VI, where a higher dose of Racecadotril (200 mg/kg) was used. The marked reduction in ulcer severity in this group highlights a dose-dependent effectiveness of Racecadotril, providing stronger evidence of its potential utility in managing indomethacin-induced gastric ulcers. This dose-response relationship is critical for understanding the therapeutic scope and ceiling of Racecadotril in clinical settings. Group VIII, treated with ranitidine, a standard H_2 receptor antagonist, displayed the most significant reduction in both erosions and inflammation. This aligns well with the established role of ranitidine in reducing gastric acid secretion, thereby protecting the gastric lining from acid-mediated damage and supporting its continued use as a benchmark treatment in gastric ulcer management.

Group III, which underwent pyloric ligation alone, displayed significant ulcerative lesions. This outcome underscores the impact of mechanically induced stress on gastric mucosa, which can lead to substantial damage due to increased gastric acid accumulation and consequent erosion of the protective mucosal barrier.

Group IV, treated with a lower dose of Racecadotril (100 mg/kg) along with pyloric ligation, showed a reduction in the number and severity of gastric lesions compared to Group III. This suggests that even at lower doses, Racecadotril has the potential to mitigate some of the damaging effects of pyloric ligation, likely through its ability to modulate gastric secretion and perhaps through protective mechanisms involving the enhancement of mucosal defenses. Further improvements were observed in Group VII, where Racecadotril was administered at a higher dose (200 mg/kg). This group displayed even greater mucosal preservation, indicating a clear dose-response relationship. The enhanced protective effect at higher doses points to the potential for optimizing Racecadotril's dosage in clinical settings for achieving maximal therapeutic benefits without incurring significant side effects. Group IX, which received standard treatment with ranitidine as compare to CMC + PL (Group III), exhibited the pronounced protection against gastric lesions. The minimal visible damage in this group reinforces the efficacy of ranitidine in reducing gastric acid secretion, which is a primary factor in the development and exacerbation of gastric ulcers. This group serves as a control demonstrating the effectiveness of conventional anti-secretory therapy, providing a benchmark against which the effects of Racecadotril can be evaluated. These findings not only validate the protective effects of standard antiulcer medications like ranitidine but also highlight the potential of Racecadotril as an alternative or adjunct treatment in managing conditions characterized by gastric hyper secretion and stress-induced mucosal damage. The results from Groups IV and VII, in particular, suggest that Racecadotril, especially at higher dosages, could be considered as a viable option in the prevention or management of gastric ulcers, particularly in patients where traditional NSAIDs and mechanical stressors are contributory factors.

4. Discussion

The present study evaluated the gastroprotective potential of Racecadotril against gastric ulcers induced by pyloric ligation and indomethacin in Sprague Dawley rats. Gastric ulcers arise primarily due to an imbalance between mucosal defense mechanisms and aggressive factors, notably increased gastric acid secretion and reduced mucosal protection associated with NSAIDs use (Khan *et al*., 2023). Pyloric ligation further exacerbates this condition by causing acid accumulation in the stomach, thereby intensifying mucosal injury (Miorando *et al*., 2024). Our findings demonstrate significant protective effects of Racecadotril in this dual-model ulcer induction scenario.

Macroscopic and quantitative evaluations of gastric lesions revealed that the group exposed to indomethacin and pyloric ligation without treatment (Indo + PL) exhibited the most severe ulceration, consistent with prior studies indicating enhanced mucosal damage due to synergistic effects of NSAIDs and gastric acid accumulation (Khan *et al*., 2024; Tan *et al*., 2023). Racecadotril, administered at two distinct doses (100 mg/kg and 200 mg/kg), showed dose-dependent protection by significantly reducing the ulcer index and visibly decreasing lesion severity compared to untreated ulcer groups. This suggests that Racecadotril possesses gastro protective properties potentially through its antisecretory activity, aligning with the known role of enkephalinase inhibition in reducing intestinal hyper secretion (Szymaszkiewicz *et al*., 2019).

Our observations on gastric acidity further support the acid-inhibitory capability of Racecadotril. The notable increase in gastric juice pH in Racecadotril-treated groups, particularly at the higher dose, indicates effective suppression of gastric acid secretion, a critical factor in ulcer pathogenesis. This finding positions Racecadotril as a possible alternative to traditional anti-secretory medications like ranitidine, which also exhibited strong anti-secretory and protective effects in our study. The reduction in gastric juice volume further corroborates the anti-secretory action of Racecadotril, suggesting potential benefits in managing hyper secretory gastric conditions.

Evaluation of gastric wall mucus revealed substantial preservation of mucus content in Racecadotril-treated animals, comparable to that observed in ranitidine-treated groups. Gastric mucus is essential for forming a protective barrier against acidic gastric secretions, and its preservation directly correlates with enhanced mucosal integrity and reduced ulceration risk (Yandrapu *et al*., 2015; Paredes *et al*., 2025). The increase in mucus content in treated groups suggests Racecadotril might stimulate mucosal defense mechanisms, thereby exerting its protective effects through multiple pathways, including enhancing mucosal barrier function.

Biochemical analyses of gastric tissues provided further insights into the gastro protective mechanisms of Racecadotril. Oxidative stress markers, notably thiobarbituric acid reactive substances (TBARS), were significantly elevated in untreated ulcer-induced groups, reflecting lipid peroxidation and oxidative mucosal injury (Omeonu *et al*., 2024). Racecadotril treatment effectively reduced oxidative damage, indicated by lower TBARS levels, and simultaneously elevated antioxidant enzymes such as Glutathione (GSH), Catalase (CAT) and Superoxide Dismutase (SOD). These enzymes play a crucial role in neutralizing reactive oxygen species (ROS) and preventing oxidative injury (Javed *et al.,* 2025), further supporting the protective role of Racecadotril through anti-oxidative pathways (Alotaibi *et al*., 2024).

The comparative effectiveness of Racecadotril and standard treatment (ranitidine) highlights Racecadotril's potential as an alternative or adjunct therapy for gastric ulcers. Given the limitations of conventional therapies, including possible adverse effects and incomplete protection against ulcer recurrence, Racecadotril's different mechanism of action could provide therapeutic advantages by offering an additional protective dimension beyond simple acid suppression.

5. Conclusion

This study investigated the acid-inhibitory and gastro protective effects of Racecadotril in an indomethacin plus pyloric ligation-induced gastric ulcer model in Sprague Dawley rats. Racecadotril, at doses of 100 mg/kg and 200 mg/kg, significantly reduced ulcer index, increased gastric pH, decreased gastric juice volume, and restored mucus content compared to the ulcer-induced groups (II). Racecadotril also decreased oxidative stress markers (TBARS) and improved antioxidant enzyme levels (GSH, CAT, SOD). The protective effects of Racecadotril were dose-dependent and comparable to the standard treatment with ranitidine. These findings suggest that Racecadotril exhibits significant gastroprotective properties through acid suppression, mucus preservation, and antioxidant enhancement, making it a promising alternative for the management of gastric ulcers.

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Availability of data and materials

All data generated or analyzed during this study are present in this article. All information is collected from the research search engines like PubMed, SciHub, MEDLINE, Sci Finder and Google Scholar.

Conflict of interest

The authors declare no conflict of interest relevant to this article.

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