

## Original Research Article

# Evaluation of anti-ulcer activity of 4-hydroxy benzaldehyde against NSAIDs induced ulcers in rats

Rupali V. Jadhav\*, V. K. Redasani, Shankar B. Kalbhare, Karishma Yadav,  
Aryan Langeh, Amuruta Tidke

Department of Pharmacology, YSPM's, Yashoda Technical Campus, Satara, Maharashtra, India

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**\*Correspondence:**

Dr. Rupali V. Jadhav,

E-mail: [rupalipbhoite@gmail.com](mailto:rupalipbhoite@gmail.com)

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### ABSTRACT

**Background:** The aim of this study was to evaluate antiulcer activity of 4-hydroxybenzaldehyde against NSAIDs induced ulcer in rats based differences in its morphology, distance with other external landmarks and also to sigmoid and transverse sinuses.

**Methods:** The antiulcer activity of 4-HBD was evaluated using pylorus ligation-aspirin induced ulcer method. Animals of this models were treated with 4-HBD (50mg/kg, 100mg/kg and 150mg/kg).

**Results:** It has been observed that 4-HBD at low dose (50mg/kg), intermediate dose (100mg/kg) and high dose (150mg/kg) showed significant increase in pH, significant decrease in gastric volume, significant decrease in ulcer index and significant decrease in total acidity.

**Conclusions:** The impact of 4-HBD therapy with intermediate (100mg/kg, p.o.) dose was observed to be similar with the positive control group.

**Keywords:** Anti-inflammatory, Antioxidant, NSAIDs, Peptic ulcers, Pylorus ligation, Wound healing

### INTRODUCTION

Peptic ulcer is nothing but lesions induced due to accumulation of acid in digestive track which is positioned in stomach or proximal duodenum. Risk factors for developing peptic ulcer include *H pylori* infection, alcohol, tobacco consumption, use of non-steroidal anti-inflammatory drugs (NSAIDs), and Zollinger-Ellison syndrome. The main risk factors for development of for both the gastric as well as duodenal ulcer are *H pylori* infection and excessive use of NSAIDs.<sup>1</sup>

Peptic ulcer disease (PUD) is the commonest disease can be seen all over the world. There are different possibilities behind the development of PUD, but the most important are *Helicobacter pylori* infection and

nonsteroidal anti-inflammatory drugs (NSAIDs). It is the type of wound in the lesion that are most commonly affected in younger to older adults population. PUD is nothing but the mucosal break of the upper gastrointestinal region due to acid peptic digestion resulting in ulcer formation which extends beyond muscularis mucosa into the submucosa. The size of the ulcer ranges from 5 mm to several cm. The term "peptic" in peptic ulcer comes from the hormone pepsin that play essential role in mucosal breakdown.<sup>2,3</sup> The drug Hydroxybenzaldehyde is of natural origin will be used as a prophylactic remedy for treating the peptic ulcer induced by NSAIDs.<sup>4,5</sup> Therefore, current study focuses on the comparative effects of 4-hydroxy benzaldehyde shows progressive anti-ulcer activity against NSAID's induced in rats. According to literature review the drug has following properties

### **Anti-inflammatory action**

4-HBD suppresses production of nitric oxide and induction of iNOS as well as COX-2 enzyme. Also diminish reactive oxygen species so this drug may be used in treatment of tissue damage due to NSAID-induced ulcers.<sup>6</sup>

### **Antioxidant property**

4-HBD affects lipid peroxidation due to NSAIDs and suppresses oxidative stress induced by reactive oxygen species that are released due to tissue damage done by NSAIDs.<sup>4</sup>

### **Wound healing action**

4-HBD promote keratinocyte migration and wound healing in mice through MAP kinase pathway so it may have contribution in treating ulcer through re-epithelization. Hence, present study was conducted to evaluate antiulcer activity of 4-HBD.<sup>7</sup>

## **METHODS**

In this study the experimental models used is pylorus ligation-aspirin induced model. The model is widely accepted for assessing antiulcer activity. Study animals were kept deprived of food for 48 hours and supplied with water. animals of control group were supplied with CMC solution (10 ml/kg), animals of negative group were treated with aspirin (100 mg/kg) and animals of positive control group were treated with pantoprazole (1.5 mg/kg) and animals from test groups were treated with low (50 mg/kg), intermediate (100 mg/kg) and high (150 mg/kg) dose of 4-hydroxybenzaldehyde respectively.<sup>6,8</sup>

### **Animals**

This prospective comparative study was conducted at Department of Pharmacology, YSPM's Yashoda Technical Campus, Wadhe, Satara, Maharashtra, India. Healthy wistar albino rats weighing about 150-200gm were used for present study. The study duration was July 2020 to March 2021. These animals were procured from registered breeder and acquainted in the quarantine area for one week.<sup>9</sup>

### **Housing of animals**

The animals were placed in polypropylene cages having paddy husk as bedding. The animals were housed under standard laboratory conditions of 22±2°C temperature, 50±15% of relative humidity, 12 hour dark/ 12 hours light) cycle with free access to pellet diet and water provided ad libitum. The experiments was performed as per as guidelines of the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Governments of India. The Institutional

Animal Ethics Committee approved the study protocol YSPM/YTC/PHARMA-IAEC/49/2020.

### **Treatment group**

Animals were randomly assigned into six group (n=6) and kept deprived of food for 48hrs.withith adequate supply of drinking water.

#### *Group 1: Control*

Healthy rats were administered with 1% CMC solution (10ml/kg).

#### *Group 2: Negative control*

Animals were administered with aspirin having a pylorus ligated stomach.

#### *Group 3: Positive control*

Animals were administered with pantoprazole (1.5mg/kg).

#### *Group 4: Test group with low dose*

Animals were administered with 4-HBD (50mg/kg).

#### *Group 5: Test group with intermediate dose*

Animals were administered with 4-HBD (100mg/kg).

#### *Group 6: Test group with high dose*

Animals were administered with 4-HBD (150mg/kg).

#### *Pylorus ligation+Aspirin induced ulcer*

This model was used to assess the antiulcer activity of drugs. Animals were kept deprived of food for 48hrs.on the next subsequent day pylorus ligation was done and animals were treated with aspirin 100 mg/kg after 5 hrs of inducer treatment animals were scarified by decapitation. Stomach was removed. Gastric volume, total acidity, pH and ulcer index was determined<sup>5</sup>.

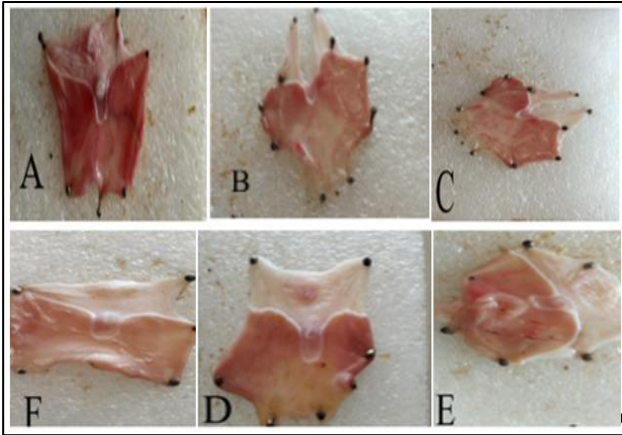
### **Statistics**

The statistical analysis was performed by using Graph pad software version 5.0 and results were compared by one-way ANOVA along with by Tukey's Multiple Comparison Test. A p value <0.05 was considered as statistically significant.<sup>5</sup>

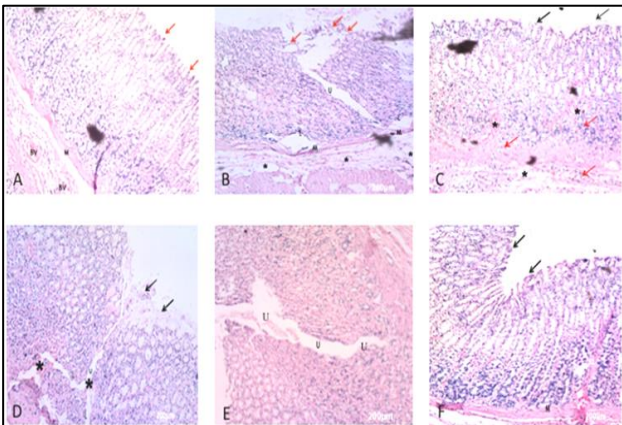
## **RESULTS**

Histopathological study of stomach was taken from scarified animal, fixed in 20% formalin solution and embedded in paraffin. Five µg sections were cut from the

paraffin block and stained with peridic acid schiff (PAS) stain for histopathological examination and ulcer protective was shown in Figure 1 to Figure 3. 4-HBD showed significant decrease in gastric acid volume ( $p<0.001$ ) at the doses of 50 mg/kg p.o., 100mg/kg p.o., and 150 mg/kg p.o. as shown in the Figure 4.



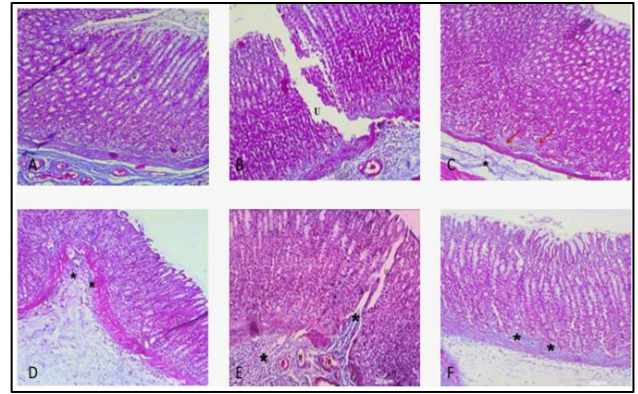
**Figure 1: Gross appearance of the gastric mucosa in treated group with (A) 4HBD, 50 mg/kg; (B) 4HBD 100 mg/kg; (C) 4HBD 150 mg/kg; (D) pantoprazole; (e) aspirin; (f) control group.**



**Figure 2: Photometric of a section in fundic mucosa in treated group with (A) 4HBD, 50 mg/kg; (B) 4HBD, 100 mg/kg; (C) 4HBD, 150 mg/kg; (D) pantoprazole 1.5 mg/kg; (E) aspirin 100 mg/kg; (F) CMC (control group).**

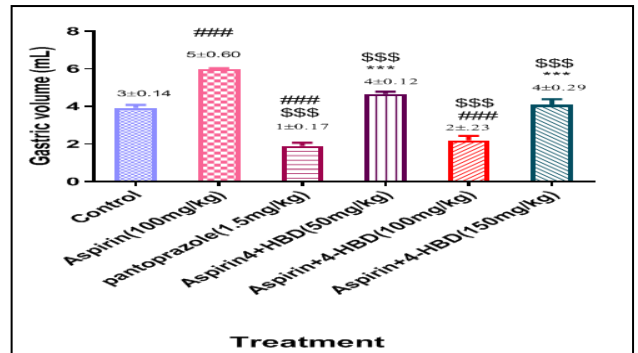
Each group was assessed at 400× magnification (H&E, scale bar=200 μm); ": clear boundaries, M: muscularis mucosa, BV: congested blood vessels in submucosa, L: loss of some cells in the basal part of the fundic gland, \*: submucosalodema and U: gastric ulcer.

4-HBD showed significant increase in gastric acid pH ( $p<0.001$ ) at the doses of 50 mg/kg p.o., 100 mg/kg p.o., and 150 mg/kg p.o. as shown in the Figure 5. 4-HBD showed significant decrease in total acidity ( $p<0.001$ ) at the doses of 50 mg/kg p.o., 100 mg/kg p.o., and 150mg/kg p.o. as shown in the Figure 6.



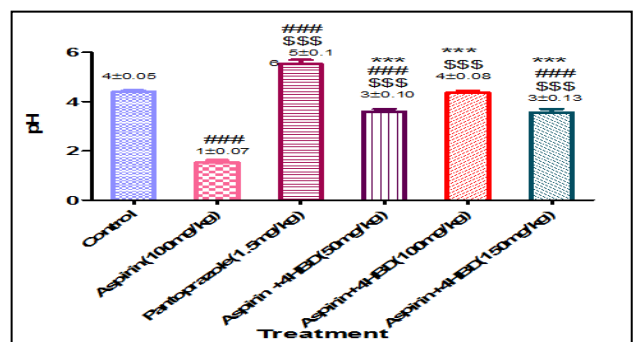
**Figure 3: Histological section of gastric mucosa in pretreated group with: (A) 4HBD, 50 mg/kg ; (B) 4HBD, 100 mg/kg b.w.; (C) 4HBD, 150 mg/kg; (D) pantoprazole, 1.5 mg/kg; (E) aspirin 100mg/kg; (F) CMC (control group).**

Each group was assessed at 400× magnification (H&E, scale bar=200 μm); ": clear boundaries, M: muscularis mucosa, BV: congested blood vessels in submucosa, L: loss of some cells in the basal part of the fundic gland, \*: submucosalodema and U: gastric ulcer.



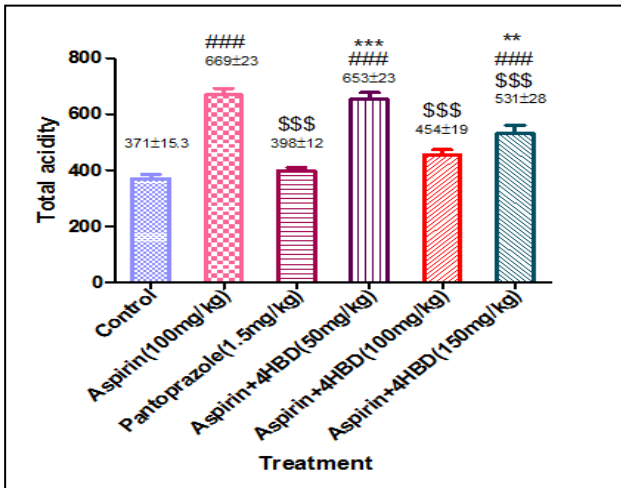
**Figure 4: Effect of 4HBD on gastric volume.**

All values are presented as mean ± SEM. Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test. # indicates comparison with control group, \$ indicate comparison with aspirin treated group, \* indicate comparison with pantoprazole. \*/\$/# indicate  $p<0.05$ , \*\*/\$\$/## indicate  $p<0.01$  and \*\*\*/\$\$/### indicate  $p<0.001$



**Figure 5: Effect of 4HBD on pH.**

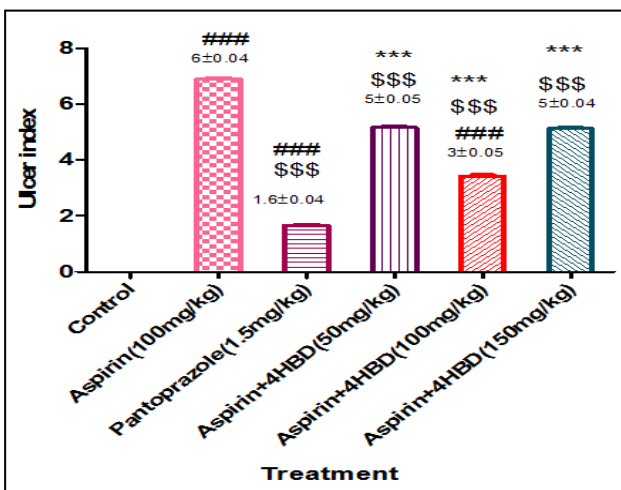
All values are presented as mean  $\pm$ sem. Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test. # indicate comparison with control group. \$ indicate comparison with aspirin treated group,\*indicate comparison with pantoprazole. \*/\$/# indicate  $p < 0.05$ , \*\*/\$\$/### indicate  $p < 0.01$  and \*\*\*/\$\$\$/#### indicate  $p < 0.001$



**Figure 6: Effect of 4-HBD on total acidity.**

All values are presented as mean  $\pm$ sem. Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test. # indicates comparison with control group. \$ indicate comparison with aspirin treated group,\*indicate comparison with pantoprazole. \*/\$/# indicate  $p < 0.05$ , \*\*/\$\$/### indicate  $p < 0.01$  and \*\*\*/\$\$\$/#### indicate  $p < 0.001$

4-HBD showed significant decrease in ulcer index ( $p < 0.001$ ) at the doses of 50 mg/kg p.o, 100 mg/kg p.o, and 150 mg/kg p.o as shown in the Figure 7.



**Figure 7: Effect of 4HBD on ulcer index.**

All values are presented as mean  $\pm$ sem. Analysis was performed using one way ANOVA followed by dTukey's multiple comparison test. # indicate comparison with control group. \$ indicate comparison with aspirin treated group,\*indicate comparison with pantoprazole. \*/\$/# indicate  $p < 0.05$ , \*\*/\$\$/### indicate  $p < 0.01$  and \*\*\*/\$\$\$/#### indicate  $p < 0.001$ .

## DISCUSSION

Peptic ulcer is one of the common diseases in human population. Due to rapid development and civilization constraints including a stressed lifestyle, the incidences of peptic ulcer are increasing.<sup>10</sup> The formation of peptic ulcers depends on the presence of acid and peptic activity in gastric juice plus a breakdown in mucosal defense.<sup>11</sup> When the equilibrium between the corrosive hydrochloric acid and acid-neutralizing mucous, which forms a protective layer on the mucosal surface, is shifted in favor of hydrochloric acid, self-digestion occurs. Various hypotheses have been proposed to understand the biochemical changes taking place during ulcer generation. Reactive oxygen species plays a role in gastric damage induced by ischemia and reperfusion, frequent use of NSAIDs, hemorrhagic shock and ethanol administration.

NSAIDs, like indomethacin, cause mucosal damage by interfering with PG synthesis, thus increasing acid secretion and the back diffusion of H<sup>+</sup> ion and resulting on overproduction of leukotrienes and other products of the 5-lipoxygenase pathway. It is generally accepted that the ulcerogenic activity of NSAIDs is related to their ability to inhibit endogenous PG synthesis by blocking COX-1 and COX-2. ROS plays an important role in mucosal damage by indomethacin. Those cause neutrophil infiltration in to stomach tissue.<sup>8</sup>

Aspirin causes mucosal damage by interfering with prostaglandin (PGE<sub>2</sub>) synthesis. This increases acid secretion and back diffusion of H<sup>+</sup> ions. Aspirin increases gastric mucosal iNOS activity. It also increases plasma concentration of TNF alpha and IL-1beta. The inflammation induced by aspirin is accompanied by increased TNF-alpha production which leads to superoxide generation and neutrophil accumulation. Ligation of the pyloric end of the stomach causes accumulation of gastric acid in the stomach. This increase in the gastric acid secretion causes ulcer in the stomach.

In NSAIDs -pylorus ligation induced gastric ulcer model the test drug 4-Hydroxybenzaldehyde attenuated the gastric volume, total acidity and ulcer index and has raised the pH value thus showing the wound healing and antioxidant property. Ulcer index parameter was used for the evaluation of anti-ulcer activity since ulcer formation is directly related to factors such as reduction in gastric volume, decrease in total acidity. The standard drug pantoprazole plays an important role in significantly reducing about 90% of ulcer induced by NSAIDs. It act as a proton pump inhibitor by blocking H<sup>+</sup>K<sup>+</sup> ATPase pump this effect leads to inhibition of both basal and stimulated gastric acid secretion. Acid volume is amount (in ml) of acid release in the gastric content release contain HCl, pepsinogen enzyme, mucus secretion, bicarbonates concentration, intrinsic factor and proteins. Amount of acid release is an important factor responsible for the production of ulcer mediated by exposure of the

unprotected lumen of stomach by concentrated acids. 4-HBD treatment showed decrease in the acid volume of the gastric secretion.<sup>12</sup> Increased pH shows a lower concentration of the hydrogen ion. The hydrogen ion is a major triggering factor responsible for the etiologic factor for ulcer and gastric damage. 4-HBD treatment indicates higher concentration of pH of the gastric juices. This value directly shown the 4-HBD reduces possibility of ulcer and has a protective effect of surface of the gastric mucosa. The present study has evaluated the antioxidant potential, anti-inflammatory and antiulcer effect of 4-HBD. The results analyzed from the present study have indicated that 4-HBD possesses antioxidant, anti-inflammatory and antiulcer effect on NSAID induced ulcers. Pre-treatment with 4-HBD particularly at a dose of 100mg/kg in a single schedule reduce the ulcer index value, total acidity concentration, total volume of acid release and increase value pH when compared with control groups. In pylorus ligation-aspirin induced model, oral administration of 4-HBD showed significant reduction in ulcer index, total acidity, gastric volume and significant rise in gastric pH at an intermediate dose (100 mg/kg) in comparison to control. In this study, we have investigated the effect of 4-HBD on experimentally induced ulcers on animal model. The drug 4-Hydroxybenzaldehyde is of natural origin will be used as a prophylactic remedy for treating the peptic ulcer induced by NSAIDs.

## CONCLUSION

The present studies suggests that the drug 4-Hydroxybenzaldehyde possesses good anti-ulcer activity which might be due to its antioxidant, anti-inflammatory and wound healing properties. The drug 4-hydroxybenzaldehyde at a intermediate dose of 100mg/kg was administered orally which prevented formation of peptic ulcer and gastric damage induced by non-steroidal anti-inflammatory agents like aspirin and indomethacin and damage due to ligation of pyloric sphincter. The drug effectively cures NSAIDs induced ulcer by decreasing gastric acid secretion, ulcer index, and total acidity and by increasing pH of gastric acid secretion. Thus, present data indicates that 4-hydroxybenzaldehyde is a safe antiulcer agent and could be used as a prophylactic agent for the treatment of peptic ulcer. An extensive study on these lines is required in future to strength the antiulcer activity of 4-hydroxybenzaldehyde as a novel antiulcer agent.

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*Conflict of interest: None declared*

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

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