

## Original Article

# Betaine improves gastroprotective effects of ranitidine and omeprazole against Indomethacin-induced gastric ulcer in rats

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## Abstract

**Background and Aim:** Antioxidant capacity of betaine has been indicated in our recent studies. Thus, we examined oral betaine as an antioxidant agent in combination with antisecretory drugs to prevent indomethacin-induced gastric damages in rats.

**Materials and Methods:** Fifty-six adult male Sprague–Dawley rats were divided into two controls (negative and normal) and five experimental groups as follows: betaine-indomethacin (Bet.-Ind.), ascorbic acid-indomethacin (Asc.-Ind.), omeprazole-indomethacin (Ome.-Ind.), betaine-omeprazole plus indomethacin (Bet.-Ome.-Ind.) and betaine-ranitidine plus indomethacin (Bet.-Ran.-Ind.).

**Results:** The betaine pretreated groups received betaine at a dosage of 1.5% (w/w) in their diet, whereas 50 mg/kg of ascorbic acid was administered orally to the Asc.-Ind., group for 15 consecutive days. After a 24 hour fast, all the groups received 48 mg/kg of indomethacin once except for normal control group. The omeprazole and ranitidine groups also received one dose of omeprazole (10 mg/kg) and ranitidine (50 mg/kg), 120 minutes before receiving indomethacin. Histopathological findings indicated the gastroprotective effects of betaine and ranitidine in pretreated rats. Pretreatment by betaine and ranitidine increased significantly the ulcer index inhibition (%), in comparison with ascorbic acid and omeprazole (alone) treatment. Glutathione peroxidase (GPx) activity was significantly higher in the Bet.-Ran.-Ind., group as compared to the Asc.-Ind., and Ome.-Ind., treated rats. GPx activity also increased significantly in Bet.-Ind., treated rats as compared to the Asc.-Ind. group. Catalase (CAT) activity was remarkably higher in the Bet.-Ran.-Ind., treated rats than the Asc.-Ind., and Ome.-Ind., groups. TBARS concentration as a lipid peroxidation marker increased significantly in Ome.-Ind., group as compared to the Bet.-Ind., and Bet.-Ran.-Ind., treated rats.

**Conclusion:** Thus, it seems that betaine as an antioxidant agent, is able to improve the effects of ranitidine and omeprazole against indomethacin-mediated gastric damages in rats. It may also be promising in the prevention of NSAIDs side effects.

**Keywords:** Betaine, Indomethacin, Omeprazole, Ranitidine, Ulcer, Rat

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## Introduction

It is widely accepted that gastric ulcer is one of the most common gastrointestinal tract diseases (1, 2). Gastric ulcer results from an imbalance between the pro-oxidant and the antioxidant systems in favor of the former in the stomach (1, 3). Gastric cell and tissue injury associated with acute and chronic inflammation are due to the toxicity of reactive oxygen species (ROS) generated in the stomach (1, 4, 5). The two major etiological factors leading to gastric ulcer are *Helicobacter pylori* infection and non steroidal anti-inflammatory drugs (NSAIDs) consumption (2). The role of oxygen derived free radicals in the development of pathogenesis in acute experimental gastric lesions induced by NSAIDs such as indomethacin is well known (3, 6-11). In this sense, indomethacin has been indicated as an oxidative agent in the previous studies (2, 12). The involvement of ROS is well established in the pathogenesis of mucosal damage caused by indomethacin, ethanol and other agents beside the inhibition of cyclooxygenase (COX) enzymes (6, 13-18). In this regard, indomethacin inhibits the synthesis of cytoprotective prostaglandins, produced by COX-1 and COX-2 in the stomach tissue (6, 16, 19). Therefore, indomethacin acts as an oxidative inducing agent and contributes to the development of ulcers.

The recent protocols to control gastric damages include the inhibition of gastric acid secretion, promotion of gastro protection, blocking of apoptosis and stimulation of epithelial cell proliferation for effective healing (2, 20). In this regard, the antisecretory drugs such as omeprazole and lansoprazole, and histamine H<sub>2</sub>-receptor blockers such as ranitidine are extensively used to control increased acid secretion and other related disorders caused by stress, NSAIDs and *H. pylori* (2). Omeprazole and lansoprazole as proton pump inhibitors are commonly used for the therapeutic control of acid-related disorders including gastroesophageal reflux and peptic-ulcer disease (12, 21-24). Omeprazole inhibits acid secretion by irreversibly interacting with the H<sup>+</sup>-K<sup>+</sup>-ATPase, the terminal proton pump of the parietal cell in the

stomach (12, 25, 26). Although omeprazole is believed to offer its antiulcer activity through acid suppression (12, 21, 24) by inactivating the H<sup>+</sup>-K<sup>+</sup>-ATPase (24-27), very little is known regarding its antioxidant effects against oxidative damage and apoptotic cell death of the gastric mucosa during ulceration.

It was reported that betaine ameliorated oxidative stress in the stomach of ethanol-induced rats (28). Moreover, based on our reports betaine is a vital antioxidant agent (6, 29-32). However, little is known about gastroprotection effects of betaine prior treatment with omeprazole and ranitidine against gastric damages in the literature. Hence, the aim of the present study was to evaluate the protective effects of betaine concomitant with ranitidine and omeprazole against indomethacin-induced gastric ulcer with respect to changes in the ulcer index inhibition, antioxidant enzyme activities, lipid peroxidation level and histopathological findings in rats.

## Materials and Methods

Betaine (Betafin<sup>®</sup> 96 %) was obtained from Biochem (Lohne, Germany). Thiobarbituric acid (TBA) was prepared from Merck Chemical Company (KGaA, Darmstadt, Germany). The GPx kit was obtained from Randox<sup>®</sup> Company (Antrim, UK). Ranitidine and omeprazole were prepared by Chemidarou<sup>®</sup> Pharmaceutical Company (Tehran, Iran). Ranitidine and omeprazole were dissolved in distilled water before administration. Other chemicals used were of analytical grade.

### Animals

A total of fifty-six adult male Sprague–Dawley rats (weighing 200±20 g, supplied from Ahvaz University of Medical Sciences, Animal House Center, Iran) were housed in temperature-controlled conditions under a 12:12-h light/dark photocycle with food and tap water supplied *ad libitum*. The rats were treated humanely and in compliance with the recommendations of Animal Care Committee for the Lorestan University of Medical Sciences with approval number 5532. All of the experimental procedures were carried out from 8.00 am to 2.00 pm and all of the treatments were applied orally by gavage.

### Experimental design

The rats were divided into two controls: negative control and normal control as well as five experimental groups (8 rats per group). The experimental groups were as follows: betaine plus indomethacin (Bet.-Ind.), ascorbic acid plus indomethacin (Asc.-Ind.), used because ascorbic acid is a well-known antioxidant agent, omeprazole plus indomethacin (Ome.-Ind.), betaine-omeprazole plus indomethacin (Bet.-Ome.-Ind.) and betaine-ranitidine plus indomethacin (Bet.-Ran.-Ind.). The control groups received 0.5 ml of normal saline daily, while the Asc.-Ind., group received 50 mg/kg BW of ascorbic acid orally for 15 consecutive days. The betaine pretreated groups (Bet.-Ind., Bet.-Ome.-Ind., and Bet.-Ran.-Ind.) received betaine at a dosage of 1.5% (w/w) in their diet for 15 consecutive days. All of the groups were kept fasting for 24 hours but had free access to water. Thereafter, indomethacin (48 mg/kg BW as orally) was then given to all groups by gavage except for the normal control group. The Ome.-Ind., Bet.-Ome.-Ind., and Bet.-Ran.-Ind., groups also received one dose of omeprazole (10 mg/kg BW) and ranitidine (50 mg/kg BW), 120 minutes, before the administration of indomethacin, respectively. Four hours after indomethacin administration, all of the groups were sacrificed upon light diethyl ether anesthesia (Dagenham, UK) by decapitation. Doses of indomethacin, ascorbic acid and omeprazole were determined according to the recent studies (12, 33), but those of betaine and ranitidine were determined based on our previous works (6, 29-32). The stomachs were removed, inflated by injecting 2 ml of normal saline and opened along a greater curvature. They were gently rinsed with normal saline to remove gastric content and blood clots before scanning. The ulcer index ((UI= (ulcerated area (mm<sup>2</sup>)/total stomach area (mm<sup>2</sup>)) ×100)), was determined by a digital camera (Panasonic WV-GP240/G, Suzhon, China), and measured according to the method previously described (34). Thereafter, ulcer index inhibition (% inhibition = (1- (UI pretreatment/UI non-pretreatment) ×100)), was evaluated based on the pretreatment by betaine, ascorbic acid, omeprazole, and ranitidine versus negative control group (**Fig. 2**). The gastric tissues were removed for biochemical

and histopathology analysis from the antral portion of the stomachs. The segments for biochemical analysis were stored at -70°C up to two months for the determination of antioxidant status and lipid peroxidation.

### Histopathological Assessment

The gastric samples of the experimental groups were processed routinely for paraffin embedding. The sections were cut at 5 µm thicknesses and stained by hematoxylin and eosin. They were then viewed under a light microscope to detect eventual histopathological changes including epithelial surface necrosis, pit cellular necrosis and glandular region necrosis. The damages varied from surface extended to glandular region. They were scored using a 0.0 through 3 grading systems as follows: 0.0=no lesion; <1=mild; 1-<2=moderate, 2-3=severe. The semiquantitative evaluation of gastric damages were stated in the Table 1 and shown in Figure 1.

### Tissue preparation for biochemical analysis

The samples were thawed and manually homogenized in a cold phosphate buffer (0.1 M, pH 7.4, containing 5 mM EDTA) on liquid nitrogen (1), and debris was removed by centrifugation at 400 g for 5 minutes (Centrifuge 5415 R; Rotofix 32A, Germany). The supernatants were recovered and used for protein measurement, antioxidant enzymes activities and TBARS concentration. The protein content of tissue homogenates was determined using a colorimetric method of Lowry with bovine serum albumin as a standard (35).

### Measurement of GPx activity

The activity of glutathione peroxidase (GPx) was evaluated with Randox<sup>®</sup> GPx detection kit according to the manufacturer's instructions as described previously (1). GPx catalyzed the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase (GR) and NADPH, the oxidized glutathione (GSSG) was immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP<sup>+</sup>. The decrease in absorbance was measured spectrophotometrically (S2000 UV model; WPA, Cambridge, UK) against blank at 340 nm. One unit (U) of GPx was defined as 1 µmol of oxidized NADPH per min per milligram of tissue protein. The GPx activity was expressed as units per milligram of tissue protein (U/mg protein).

### Measurement of CAT activity

The catalase activity in tissue was assayed using the method described by Claiborne (36), as referred to previously (1). The reaction mixture (1 ml) consisted of 50 mM potassium phosphate (pH 7.0), 19 mM H<sub>2</sub>O<sub>2</sub>, and a 20–50 µl sample. The reaction was initiated by the addition of H<sub>2</sub>O<sub>2</sub> and then absorbance changes were measured at 240 nm (25°C) for 30 s. The molar extinction coefficient for H<sub>2</sub>O<sub>2</sub> was 43.6 M<sup>-1</sup>.cm<sup>-1</sup>. The CAT activity was expressed as the unit that is defined as µmol of H<sub>2</sub>O<sub>2</sub> consumed per min per milligram of tissue protein (U/mg protein).

### Measurement of lipid peroxidation

The amount of lipid peroxidation was indicated by the content of thiobarbituric acid reactive substances (TBARS) in the stomach. Tissue TBARS was determined by following the production of thiobarbituric acid reactive substances as described previously (1, 37). In short, 40 µl of homogenate was added to 40 µl of 0.9% NaCl and 40 µl of deionized H<sub>2</sub>O, resulting in a total reaction volume of 120 µl. The reaction was incubated at 37° C for 20 minutes and stopped by the addition of 600 µl of cold 0.8 M hydrochloric acid, containing 12.5% trichloroacetic acid. Following the addition of 780 µl of 1% TBA, the reaction was boiled for 20 minutes and then cooled at 4° C for 1 hour. In order to measure the amount of TBARS produced by the homogenate, the cooled reaction was spun at 1500 g in a microcentrifuge for 20 minutes and the absorbance of the supernatant was read at 532 nm, using an extinction coefficient of 1.56×10<sup>5</sup> M<sup>-1</sup>.cm<sup>-1</sup>. The blanks for all of the TBARS assays contained an additional 40 µl of 0.9% NaCl instead of homogenate as just described. TBARS results were expressed as nanomole per milligram of tissue protein (nmol/mg protein).

### Statistical analysis

The statistical differences were applied among the pretreated groups by one-way analysis of variance (ANOVA) via Tukey's post hoc analysis. All results were presented as mean± (S.E.M.). Moreover, a calculated *P* value of less than 0.05 was considered statistically significant. Statistical analysis was performed using the statistical package of GraphPad PRISM version 5 (Graphpad Software Inc., San Diego, CA, USA). Previously, all variables were

tested for normal and homogeneous variances by *Leven's* statistic test.

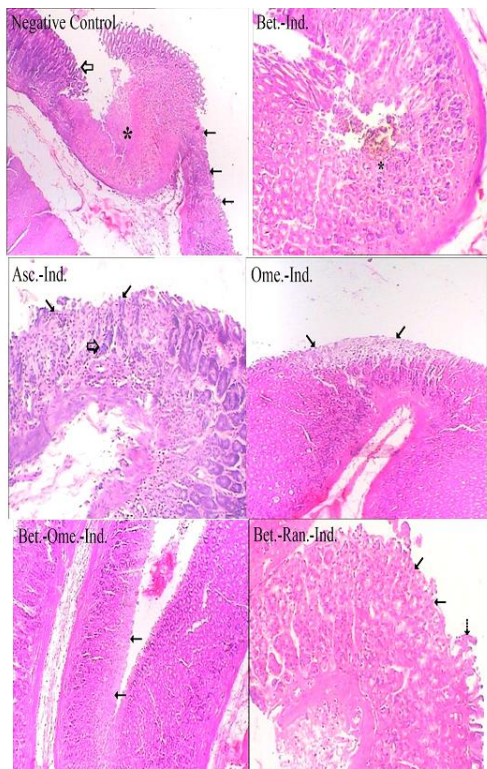
## Results and Discussion

Regarding the indomethacin-induced effects in the stomach wall, administration of indomethacin to rats produced visible gastric ulcers among the rats in negative control group, which were localized in the portion of the stomach secreting acid and pepsin (corpus part). Fasting could induce only gastric erosion in normal control group and ulcer was not observed in the stomach wall following fasting. In the pretreated rats, epithelial surface necrosis decreased significantly in the Bet.-Ind., and Bet.-Ran.-Ind., treated rats as compared to the Asc.-Ind., Ome.-Ind., and Bet.-Ome.-Ind., groups. Betaine plus omeprazole could decrease pit cellular necrosis in the Bet.-Ome.-Ind., group as compared to the Bet.-Ind., Asc.-Ind., Ome.-Ind., and Bet.-Ran.-Ind treated rats. Betaine pretreatment in Bet.-Ome.-Ind., and Bet.-Ran.-Ind., groups indicated a preventive effect on glandular necrosis in comparison with Bet.-Ind., Asc.-Ind., and Ome.-Ind., groups (Table 1). The histopathological findings of the experimental groups also confirmed the gastroprotective effects of betaine concomitant with antisecretory drugs (omeprazole and ranitidine) in rats (Figure 1).

In the experimental groups, it was observed that pretreatment by betaine and betaine plus ranitidine significantly increased the ulcer index inhibition (%), in comparison with the Asc.-Ind., Ome.-Ind., and Bet.-Ome.-Ind., groups (*P* < 0.05). Although the Bet.-Ome.-Ind., group indicated lower ulcer index inhibition (%) in comparison with the Bet.-Ran.-Ind. treated rats, the difference was not significant (*p* > 0.05; Figure 2).

GPx activity was significantly lower in the Asc.-Ind., group compared to the Bet.-Ind., treated rats (*P* < 0.05). In contrast, GPx activity was significantly higher in the Bet.-Ran.-Ind., group as compared to the Asc.-Ind., Ome.-Ind., and the Bet.-Ome.-Ind., treated rats (*p* < 0.05). Indeed, when ranitidine (as an antisecretory drug) was administered in combination with betaine, it could increase significantly the activity of GPx in comparison with ascorbic acid, omeprazole and even betaine plus omeprazole treatments (Figure 3). CAT activity rose significantly in the Bet.-Ran.-Ind., group

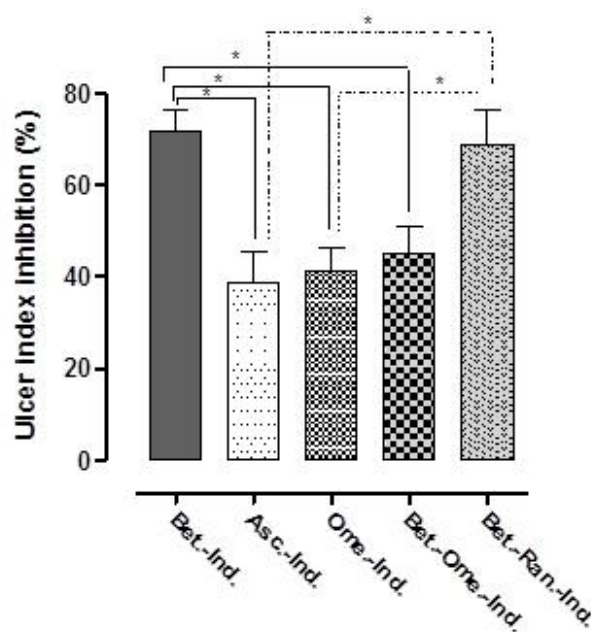




**Figure 1.** Negative control: Deep erosion penetrates through the stomach wall in the center (astrisk) and right side of the lesion (arrows). The extension of coagulative necrosis occurs through the whole thickness of the gastric mucosae but it does not breach the muscularis layer. In the left side of ulcer, the gastric epithelium is slightly intact (blank arrow). HE × 40. Betaine-Indometacin: erosion of gastric mucosa is produced by the necrosis of superficial parts of epithelium but is not extend beyond the glandular tissue (astrisk). HE × 100. Ascorbic acid-Indometacin: deep erosion of gastric mucosae (arrows). This lesion is very similar to negative control, however it might be a slightly distorted viable gland (blank arrow). HE × 100. Omeprazole-Indometacin: the necrosis is extended to superficial parts of glandular tissue (arrows). Moreover, beneath the necrotic tissue, the viable glands are deeply basophilic. HE × 40. Betaine-Omeprazole-Indometacin: dispense of normal glandular structures. The destructive process is fairly similar to Ome-Ind, in which intensive necrosis is limited at superficial regions (arrows). HE × 40. Betaine-Ranitidine-Indometacin: mild to moderate detachment of superficial layer of epithelium (arrows). In the right-bottom the mucosa is completely unchanged (dot arrow). HE × 100. Bet.; Betaine, Ind.; Indometacin, Asc.; Ascorbic acid, Ome.; Omeprazole and Ran.; Ranitidine.

as compared to the Asc.-Ind., and the Ome.-Ind., treated rats ( $p < 0.05$ , Figure 4).

Treatment of rats with omeprazole (alone) increased significantly the lipid peroxidation marker (TBARS concentration) in the gastric tissue as compared to the Bet.-Ind., and Bet.-Ran.-Ind., groups ( $p < 0.05$ ).

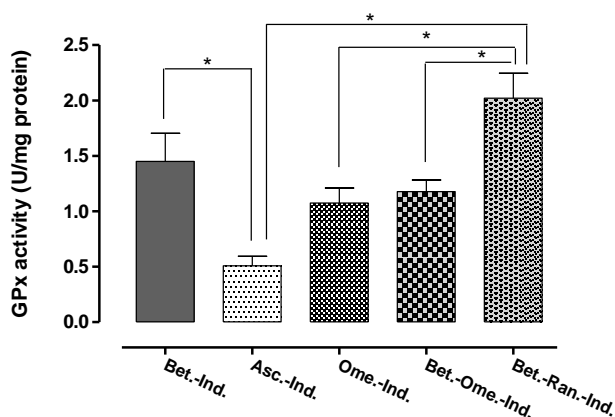


**Figure 2.** Comparison of ulcer index inhibition among the pretreated groups (Ulcer index inhibition was determined based on the pretreatment by betaine, ascorbic acid, omeprazole and ranitidine in comparison with negative control group which received only indomethacin). Values represent mean± SEM of ulcer index inhibition (%). \*Asterisk indicates statistically difference between groups (One-way ANOVA followed by Tukey's post hoc test;  $p < 0.05$ ). Bet.; Betaine, Ind.; Indometacin, Asc.; Ascorbic acid, Ome.; Omeprazole and Ran.; Ranitidine.

Pretreatment by betaine and betaine plus ranitidine could suppress TBARS concentration ( $p < 0.05$ , Figure 5).

**Discussion**

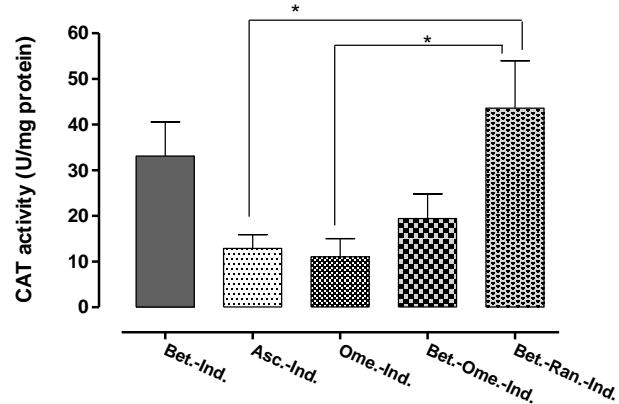
During the past decade, administration of betaine has been exerted as an antioxidant agent in oxidative stress-induced models (6, 29-32). Betaine (trimethylglycine) has been used in the form of an antioxidant and an osmolyte as a dietary feed supplement in animal nutrition (38) to protect cells, proteins and enzymes from environmental stress (39) with a wide range of oral doses (20). Betaine is also believed to play a significant role in maintaining the structural and functional integrity of cell membranes (6). In the present study, the administration of indomethacin to fasted rats resulted in severe gastric hemorrhage in negative control group as compared to normal rats. Whereas, pretreatment by betaine increased significantly the index inhibition of gastric ulcers (%) in betaine group and betaine plus ranitidine-treated rats. Ranitidine is widely known as an



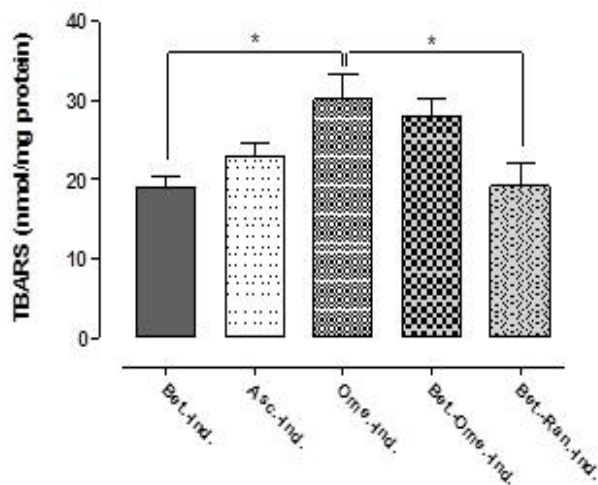
**Figure 3.** Comparison of glutathione peroxidase (GPx) activity among the pretreated groups. Values represent mean  $\pm$  SEM of enzyme activity (unit/mg protein of gastric tissue). \*Asterisk indicates statistically difference between groups (One-way ANOVA followed by Tukey's post hoc test;  $p < 0.05$ ). Bet.; Betaine, Ind.; Indomethacin, Asc.; Ascorbic acid, Ome.; Omeprazole and Ran.; Ranitidine.

antisecretory drug and is frequently used as a reference drug in experimental studies of gastric damages and in clinical practice as well (1, 40). Hence, it seems that betaine as an antioxidant agent may be beneficial in preventing direct mucosal cell damage, whereas ranitidine can be useful as an antisecretory drug for the prevention of HCl secretion in the stomach wall (1, 16). These results are in agreement with our recent study in which oleuropein (as antioxidant agent) in combination with ranitidine could prevent gastric ulcer in rats (1).

In the experimental animal models, the role of acid in gastric lesions has been studied using stress or nonsteroidal anti-inflammatory drugs (NSAIDs) consumption (12). Increased production of hydrochloric acid in the ulcerated condition might be a consequence of increased permeability of the mucosa, which is an important process in the development of ulcer (1, 2). The previous report confirmed that NSAID consumption could raise gastric acidity (2). Although omeprazole, the primary member of the proton pump inhibitors, has been used extensively to control gastric disorders (12, 21), omeprazole neither stimulates prostaglandin biosynthesis nor increases bicarbonate secretion to offer gastroprotection. Thus, omeprazole may exert its antiulcer activity through some other mechanism such as antioxidant activity. This ability was



**Figure 4.** Comparison of catalase (CAT) activity among the pretreated groups. Values represent mean  $\pm$  SEM of enzyme activity (unit/mg protein of gastric tissue). \*Asterisk indicates statistically difference between groups (One-way ANOVA followed by Tukey's post hoc test;  $P < 0.05$ ). Bet.; Betaine, Ind.; Indomethacin, Asc.; Ascorbic acid, Ome.; Omeprazole and Ran.; Ranitidine.



**Figure 5.** Comparison of the concentration of thiobarbituric acid reactive substances (TBARS) among the pretreated groups. Values represent mean  $\pm$  SEM of TBARS (nanomoles per milligram protein of gastric tissue). \*Asterisk indicates statistically difference between groups (One-way ANOVA followed by Tukey's post hoc test;  $P < 0.05$ ). Bet.; Betaine, Ind.; Indomethacin, Asc.; Ascorbic acid, Ome.; Omeprazole and Ran.; Ranitidine.

confirmed by a previous report (12). Herein, omeprazole partially offered gastroprotection through a mechanism other than acid inhibition. This was further substantiated by the finding that ulcer index inhibition in Ome.-Ind., treated rats was similar to the pretreated rats in terms of ascorbic acid as a reference antioxidant agent. However, lesions developed when omeprazole (alone) was administered prior to indomethacin. Our results indicated that omeprazole

**Table 1:** The effects of betaine, ascorbic acid, omeprazole and ranitidine on semiquantitative histopathological findings of the indomethacin-induced gastric lesions in rats.

lesions	Bet.-Ind.	Asc.-Ind.	Ome.-Ind.	Bet.-Ome.-Ind.	Bet.-Ran.-Ind.
Epithelial surface necrosis	1.60 <sup>a</sup>	2.50	2.25	2.25	0.75 <sup>d</sup>
Pit cellular necrosis	1.20	2.66	1.25	0.50 <sup>b</sup>	1.00
Glandular necrosis	0.80	1.16	1.00	0.00 <sup>c</sup>	0.00 <sup>c</sup>

The damages were from surface extended to glandular region and scored using a 0.0 through 3 grading system. 0.0 =no lesion; <1=mild; 1-<2=moderate, 2-3=severe.

<sup>a</sup> Epithelial surface necrosis was significantly less in the Bet.-Ind. and Bet.-Ran.-Ind. treated rats as compared to the Asc.-Ind., Ome.-Ind., and Bet.-Ome.-Ind., groups.

<sup>b</sup> The Bet.-Ome.-Ind., group could decrease the effects of indomethacin in case of pit cellular necrosis in comparison with Bet.-Ind., Asc.-Ind., Ome.-Ind., and Bet.-Ran.-Ind., treated rats.

<sup>c</sup> Glandular necrosis was not observed in Bet.-Ome.-Ind., and Bet.-Ran.-Ind., groups in contrast to the Bet.-Ind., Asc.-Ind., and Ome.-Ind., groups. Bet.; Betaine, Ind., Indomethacin, Asc., Ascorbic acid, Ome., Omeprazole and Ran., Ranitidine.

was less effective in preventing gastric damages induced by an oxidative agent such as indomethacin. This result was further confirmed by an elevation of lipid peroxidation and a reduction in antioxidant enzyme activities as well as histopathology findings in omeprazole (alone) pretreated rats.

It is widely accepted that lipid peroxidation is a mechanism of cellular injury (1, 6, 34, 40). Thus, we measured the TBARS concentration as an indicator of lipid peroxidation in gastric wall. Our results showed that there was a significant increase in the lipid peroxidation level in Ome.-Ind., treated rats. In contrast, significant decreases in the TBARS concentration were observed after the administration of betaine and ranitidine in Bet.-Ind., and Bet.-Ran.-Ind., groups. Hence, being pretreated by omeprazole could not prevent lipid peroxidation adequately. Interestingly, Asc.-Ind treated rats showed lower TBARS concentration in comparison with Ome.-Ind., group. In this regard, we know that vitamin C is essential for collagen biosynthesis. Ascorbate is a cofactor for prolyl and lysyl hydroxylases, the enzymes responsible for stabilizing and cross-linking collagen in connective tissue of stomach. Vitamin C also influences collagen synthesis independently of hydroxylation by activating collagen transcription factor and stabilizing procollagen messenger (mRNA) (33). Moreover, the antioxidant capacity of vitamin C is widely accepted. It could suppress lipid peroxidation induced by indomethacin. Therefore, the collagen synthesis and antioxidant capacity in the rats pretreated by vitamin C could both partially increase ulcer index inhibition and decrease lipid

peroxidation in the stomach wall of rats.

As previously mentioned, oxidative damage and apoptotic cell death play significant roles in the loss of gastric mucosal integrity caused by various ulcerogens (1, 7, 12). Indeed, ulcers develop when oxidative damage and apoptosis predominate over the healing process by cell proliferation (1, 12). Based on the results, oral administration of betaine prior to indomethacin administration attenuated hemorrhage and increased ulcer index inhibition as compared to the negative control group, which received indomethacin alone. Betaine is widely known as an antioxidant agent. Moreover, betaine, through its participation in sequential methylation within the cellular membranes, maintains a proper balance between phosphatidyl ethanolamine and phosphatidyl choline, thus sustaining proper membranes (6, 30-32, 38). Therefore, betaine could protect stomach wall in pretreated rats. Furthermore, there was a significant increase in the ulcer index inhibition in these groups as compared to the no betaine pretreated rats.

Gastric ulcer is an oxidative state wherein acid and pepsin contribute to the development of this condition. Therefore, antioxidant enzyme activities and lipid peroxidation explain the gastric oxidative-antioxidant imbalance (1). All cells are able to defend themselves against the harmful effects of oxygen free radicals through their own antioxidant mechanisms, including enzymatic and non-enzymatic antioxidant systems. GPx and CAT are two key antioxidant enzymes that can decompose hydrogen peroxide to water (6). SOD, another antioxidant enzyme in cells, rapidly converts superoxide anion (O<sub>2</sub><sup>-</sup>) to less dangerous hydrogen

peroxide (H<sub>2</sub>O<sub>2</sub>). Thereafter, GPx and CAT can decompose H<sub>2</sub>O<sub>2</sub> to water. Although, H<sub>2</sub>O<sub>2</sub> is not a particularly reactive product, it may be reduced to highly reactive metabolites hydroxyl radicals and/or single oxygen (1). Herein, betaine caused significant increases in the activities of antioxidant enzymes (GPx and CAT) for both Bet.-Ind., and Bet.-Ran.-Ind., groups in comparison with Ome.-Ind., Asc.-Ind., and Bet.-Ome.-Ind., groups. On the other hand, betaine could enhance the antioxidant status system of gastric wall via its antioxidant effects. Moreover, gastric defense was not depleted in the betaine and betaine-ranitidine treated rats. Our data suggest that betaine and ranitidine prevented the oxidative effects of indomethacin and preserved the cellular antioxidant stores. These effects of betaine and ranitidine result from their capacities to scavenge ROS and to block acid secretion, respectively (1, 6, 31).

## Conclusion

In conclusion, we observed valuable effects of betaine prior treatment by antisecretory drugs such as omeprazole and ranitidine. Although betaine could prevent oxidative effects of indomethacin principally in combination with ranitidine, it will be interesting to examine its biological effects in further studies.

## Acknowledgment

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## Conflict of Interest

The authors declare that they have no conflict of interest.

## References

- Alirezaei M, Dezfoulian O, Neamati S, Rashidipour M, Tanideh N, Kheradmand A. Oleuropein prevents ethanol-induced gastric ulcers via elevation of antioxidant enzyme activities in rats. *J Physiol Biochem.* 2012;68:583-92.
- Ganesan B, Yathavamoorthy R, Farvin KSH, Anandan R. Supplementation of betaine attenuates HCl-ethanol induced gastric ulcer in rats. *Int J Biol Chem.* 2010;4:79-89.
- Bandyopadhyay SK, Pakrashi SC, Pakrashi A. The role of antioxidant activity of *Phyllanthus emblica* fruits on prevention

from indomethacin induced gastric ulcer. *J Ethnopharmacol.* 2000;70:171-6.

- Lee JH, Lee DU, Jeong CS. Gardenia jasminoides Ellis ethanol extract and its constituents reduce the risks of gastritis and reverse gastric lesions in rats. *Food Chem Toxicol.* 2009;47:1127-31.
- Leirisalo-Repo M, Paimela L, Koskimies S, Repo H. Functions of polymorphonuclear leukocytes in early rheumatoid arthritis. *Inflammation.* 1993;17:427-42.
- Alirezaei M, Jelodar G, Niknam P, Ghayemi Z, Nazifi S. Betaine prevents ethanol-induced oxidative stress and reduces total homocysteine in the rat cerebellum. *J Physiol Biochem.* 2011;67:605-12.
- Das D, Banerjee RK. Effect of stress on the antioxidant enzymes and gastric ulceration. *Mol Cell Biochem.* 1993;125:115-25.
- De Barros MP, Sousa JPB, Bastos JK, De Andrade SF. Effect of Brazilian green propolis on experimental gastric ulcers in rats. *J Ethnopharmacol.* 2007;110:567-71.
- Hetil O. Mechanism of free radicals in gastrointestinal and liver diseases. *J Clin Biol.* 1993;134:675-83.
- Isenberg JI, McQuaid KR, Laine L, Walsh JH. Acid-peptic disorders. *Textbook of gastroenterology.* 1995;1:1.347-1.430.
- Itoh M, Guth PH. Role of oxygen-derived free radicals in hemorrhagic shock-induced gastric lesions in the rat. *Gastroenterology.* 1985;88:1165-7.
- Biswas K, Bandyopadhyay U, Chattopadhyay I, Varadaraj A, Ali E, Banerjee RK. A novel antioxidant and antiapoptotic role of omeprazole to block gastric ulcer through scavenging of hydroxyl radical. *J Biol Chem.* 2003;278:10993-1001.
- Das D, Bandyopadhyay D, Bhattacharjee M, Banerjee RK. Hydroxyl radical is the major causative factor in stress-induced gastric ulceration. *Free Radical Biol Med.* 1997;23:8-18.
- Elliott SN, Wallace JL. Neutrophil-mediated gastrointestinal injury. *Can J Gastroenterol.* 1998;12:559-68.
- Miura T, Muraoka S, Fujimoto Y. Lipid peroxidation induced by indomethacin with horseradish peroxidase and hydrogen peroxide: involvement of indomethacin radicals. *Biochem Pharmacol.* 2002;63:2069-74.
- Odabasoglu F, Cakir A, Suleyman H, Aslan A, Bayir Y, Halici M, Kazaz C. Gastroprotective and antioxidant effects of usnic acid on indomethacin-induced gastric ulcer in rats. *J Ethnopharmacol.* 2006;103:59-65.
- Smith SM, Kvietyts PR. Gastric ulcers: role of oxygen radicals. *Crit. Care Med.* 1988;16:892-8.
- Wallace JL. Mechanisms of protection and healing: current knowledge and future research. *American J Med.* 2001;110:19-23.
- De Souza GEP, Cardoso RA, Melo MCC, Fabricio ASC, Silva VMS, Lora M, De Brum-Fernandes AJ, Rae GA, Ferreira SH, Zampronio AR. A comparative study of the antipyretic effects of indomethacin and dipyron in rats. *Inflammation Res.* 2002;51:24-32.
- Ganesan B, Anandan R, Lakshmanan PT. Studies on the protective effects of betaine against oxidative damage during experimentally induced restraint stress in Wistar albino rats. *Cell Stress Chaperones.* 2011;16:641-52.
- Langtry HD, Wilde MI. Omeprazole. *Drugs* 1998;56:447-86.
- Garnett WR. Lansoprazole: a proton pump inhibitor. *Ann Pharmacother.* 1996;30:1425-36.



23. Zimmermann AE, Katona BG. Lansoprazole: a comprehensive review. *Pharmacotherapy: J Human Pharmacol Drug Therapy*. 1997;17:308-26.
24. Wolfe MM, Sachs G. Acid suppression: optimizing therapy for gastroduodenal ulcer healing, gastroesophageal reflux disease, and stress-related erosive syndrome. *Gastroenterology*. 2000;118:S9-S31.
25. Fellenius E, Berglindh T, Sachs G, Olbe L, Elander B, Sjostrand S-E, Wallmark B. Substituted benzimidazoles inhibit gastric acid secretion by blocking (H<sup>+</sup> & plus; K<sup>+</sup>)-ATPase. *Nature*. 1981;290:159-61.
26. Sachs G. Proton pump inhibitors and acid-related diseases. *Pharmacotherapy: J Human Pharmacol Drug Therapy*. 1997;17:22-37.
27. Wallmark B, Larsson H, Humble L. The relationship between gastric acid secretion and gastric H<sup>+</sup>, K<sup>+</sup>-ATPase activity. *J Biol Chem*. 1985;260:13681-4.
28. Ahn M, Kang Y, Moon J, Kim S, Moon C, Shin T. Oral administration of betaine ameliorates ethanol-induced gastric injury in rats through its antioxidant effects. *Orient Pharm Exp Med*. 2014;14:237-43.
29. Alirezaei M. Betaine as a methyl donor and an antioxidant agent in levodopa-induced hyperhomocysteinemia and oxidative stress in rat's kidney. *Iranian Journal of Veterinary Medicine* 2014;8:91-9.
30. Alirezaei M. Betaine protects cerebellum from oxidative stress following levodopa and benserazide administration in rats. *Iran J Basic Med Sci*. 2014;18:950.
31. Alirezaei M, Khoshdel Z, Dezfoulian O, Rashidipour M, Taghadosi V. Beneficial antioxidant properties of betaine against oxidative stress mediated by levodopa/benserazide in the brain of rats. *J Physiol Sci*. 2015;65:243-52.
32. Alirezaei M, Niknam P, Jelodar G. Betaine elevates ovarian antioxidant enzyme activities and demonstrates methyl donor effect in non-pregnant rats. *Int J Peptide Res Therap*. 2012;18:281-90.
33. Farris PK. Topical vitamin C: a useful agent for treating photoaging and other dermatologic conditions. *Dermatol Surg*. 2005;31:814-8.
34. Dekanski D, Janicijevic-Hudomal S, Ristic S, Radonjic NV, Petronijevic ND, Piperski V, Mitrovic DM. Attenuation of cold restraint stress-induced gastric lesions by an olive leaf extract. *Gen Physiol Biophys* 2009;28:135-42.
35. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem*. 1951;193:265-75.
36. Claiborn A. CRC handbook of methods for oxygen radical research. CRC press Boca Raton FL; 1986.
37. Subbarao KV, Richardson JS, Ang LC. Autopsy samples of Alzheimer's cortex show increased peroxidation in vitro. *J Neur*. 1990;55:342-5.
38. Alirezaei M, Gheisari HR, Ranjbar VR, Hajibemani A. Betaine: a promising antioxidant agent for enhancement of broiler meat quality. *Br Poult Sci*. 2012;53:699-707.
39. Craig SAS. Betaine in human nutrition. *Amr J Clin Nut*. 2004;80:539-49.
40. Dekanski D, Ristic S, Mitrovic DM. Antioxidant effect of dry olive (*Olea europaea* L.) leaf extract on ethanol-induced gastric lesions in rats. *Med J Nut Met*. 2009;2:205-11.
41. Evbuomwan MI, Bolarinwa Y. Role of indomethacin-induced peptic ulceration in gastric acid secretion in pregnant rats. *Afr J Med Med Sci*. 1993;22:29-31.
42. Mattsson H, Andersson K, Larsson Hk. Omeprazole provides protection against experimentally induced gastric mucosal lesions. *Eur. J. Pharmacol*. 1983;91:111-4.
43. Alirezaei M, Kheradmand A, Heydari R, Tanideh N, Neamati S, Rashidipour M. Oleuropein protects against ethanol-induced oxidative stress and modulates sperm quality in the rat testis. *Med J Nut Met*. 2011:1-7.
44. Neamati S, Alirezaei M, Kheradmand A. Ghrelin Acts as an Antioxidant Agent in the Rat Kidney. *Int J Pep Res Therap*. 2011;17:239-45.
45. Kheradmand A, Alirezaei M, Birjandi M. Ghrelin promotes antioxidant enzyme activity and reduces lipid peroxidation in the rat ovary. *Regulatory Peptides* 2010;162:84-9.
46. Turner TT, Lysiak JJ. Oxidative stress: a common factor in testicular dysfunction. *J Androl*. 2008;29:488-98.
47. Kheradmand A, Alirezaei M, Asadian P, Alavi ER, Joorabi S. Antioxidant enzyme activity and MDA level in the rat testis following chronic administration of ghrelin. *Andrologia*. 2009;41:335-40.
48. Peltola V, Huhtaniemi I, Metsa-Ketela T, Ahotupa M. Induction of lipid peroxidation during steroidogenesis in the rat testis. *Endocrinology*. 1996;137:105-12.