

Protective Effects of N-Acetylcysteine on Gastric Blood Flow, Nitric Oxide Bioavailability, Oxidative Stress, and Hematological Alterations in Indomethacin-Induced Gastric Ulceration in Male Wistar Rats

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Abstract

INTRODUCTION:

Nonsteroidal anti-inflammatory drugs (NSAIDs), particularly indomethacin, are widely associated with gastrointestinal complications, including gastric ulceration, due to disruption of gastric mucosal defense mechanisms. N-Acetyl cysteine (NAC), a sulfur-containing compound with potent antioxidant and mucolytic properties, has demonstrated promising gastroprotective effects. This study investigated the dose-dependent gastroprotective and anti-ulcer effects of NAC compared with cimetidine in an indomethacin-induced gastric ulcer model in rats.

METHODOLOGY:

A total of sixty (60) animals weighing between 150g and 180g were divided into 2 separate sets for separate experiments, with thirty (30) animals in each set. The 2 separate sets are further divided into 5 sub-groups with 6 animals in each sub-group. The 2 (two sets of animals) undergo the same experimental treatment. Group I: Rats served as control received distilled water orally for 7 days before induction of ulcer with indomethacin (40 mg/kg) (ulcer control). Group II: Rats received cimetidine orally for 7 days (50 mg/kg) and indomethacin orally (40 mg/kg) on the last day. Group III, IV, V: Rats received NAC at doses of 100, 300, and 500 mg/kg, respectively, for 7 days orally before indomethacin orally (40 mg/kg) on the last day. One experimental set was used for the assessment of gastric blood flow and antioxidant parameters, while the second set was used for the evaluation of gastric mucus secretion. Following sacrifice by cervical dislocation, biological samples were collected for biochemical analyses.

RESULTS

The findings from this study indicate that an increase in gastric blood flow was observed in all treated groups, with the most notable increases in the groups treated with 300 mg/kg and 500 mg/kg cysteine, compared to the ulcer control. Sulfhydryl concentration also rose in all treated groups, particularly in the group receiving 500 mg/kg cysteine, relative to the ulcer control. The antioxidant enzyme SOD significantly increased across all treated groups, with the highest levels seen in the 500 mg/kg cysteine group, compared to the ulcer control. Mucus secretion was notably enhanced in all treated groups, especially in the 500 mg/kg cysteine group, relative to the control. The above results demonstrate the role of NAC in ameliorating indomethacin-induced gastric ulceration.

Introduction

Peptic ulcer disease (PUD), also known as a peptic ulcer or stomach ulcer, is a break in the lining of the stomach, the first part of the small intestine, or occasionally the lower esophagus [1]. Peptic ulcer, which is a common condition that causes a high rate of mortality in humans worldwide, is a multifactorial and

complex disease involving gastric and duodenal ulcers [2]. The pathogenesis of peptic ulcer disease is multifactorial and involves disruption of the physiological balance between mucosal defensive factors and injurious stimuli [3]. Major risk factors include chronic NSAID use, *Helicobacter pylori* infection, excessive alcohol consumption, cigarette smoking, psychological stress, genetic predisposition, and advancing age [4]. It is well known that peptic lesions arise from an imbalance between aggressive and defensive factors [5]. Therapeutic agents for peptic ulceration have therefore been developed to mitigate the aggressive factors or reinforce the defensive factors. The approach most frequently used worldwide is to decrease the production of the aggressive factors, acid and pepsin, by administering histamine H₂-receptor antagonists or H⁺-K⁺ ATPase inhibitors [6]. Moreover, the strong inhibitors of gastric acid secretion, the symptoms of gastric ulcer, as a type of peptic ulcer, include belching, vomiting, weight loss, or poor appetite. Approximately one-third of older people experience no symptoms. Complications may include bleeding, perforation, and blockage of the stomach [7].

The stomach is the first place where various substances ingested into the body goes especially the gastric mucosa lining, which is always exposed to harmful substances like food, chemicals, pepsin, acid, and bacteria, especially *Helicobacter pylori* [8]. All these could, in a way, be harmful to the stomach, thereby leading to a gastric ulcer. Gastric ulcers occur within the stomach, whereas duodenal ulcers develop in the proximal portion of the small intestine [9]. The disease arises from a complex interaction between aggressive factors, such as gastric acid, pepsin, *Helicobacter pylori* infection, and non-steroidal anti-inflammatory drugs (NSAIDs), and protective mechanisms including mucus and bicarbonate secretion, adequate mucosal blood flow, prostaglandin synthesis, nitric oxide production, antioxidant defense systems, and epithelial regeneration [10].

NSAIDs such as indomethacin or aspirin are known to be prescribed drugs for the treatment of pain and inflammation in rheumatic disorders and osteoarthritis, hence the side effects resulting in organ damage [11]. Indomethacin, a potent non-selective NSAID, is widely employed in experimental models of gastric ulcer because it reliably reproduces the pathological features of NSAID-induced mucosal injury observed in humans [12, 13]. The ulcerogenic effects of indomethacin are mediated through both cyclooxygenase (COX)-dependent and COX-independent pathways. Inhibition of COX enzymes suppresses prostaglandin synthesis, leading to reduced mucus and bicarbonate secretion, impaired gastric blood flow, and diminished epithelial repair. Additionally, indomethacin promotes oxidative stress, neutrophil infiltration, mitochondrial dysfunction, inflammatory cytokine production, and lipid peroxidation, all of which contribute to gastric mucosal damage [14].

Current pharmacological management of peptic ulcer disease primarily focuses on reducing gastric acid secretion through histamine H₂ receptor antagonists, proton pump inhibitors (PPIs), and eradication of *H. pylori* infection, where indicated [15]. Cimetidine is in the class of the first-generation histamine H₂ receptor antagonist, alongside ranitidine and famotidine, that inhibits stomach acid production [16]. It is mainly used sometimes in the treatment of heartburn and often to treat peptic ulcers. Cimetidine is used to reduce the pain of ulcers and heartburn or to assist in the healing of ulcers and damage caused by gastroesophageal reflux disease (GERD) [17]. Cimetidine is also used to prevent ulcers in certain

circumstances by treating certain conditions that are associated with hypersecretion of acid in the stomach (i.e., Zollinger-Ellison Syndrome, systemic mastocytosis, and multiple endocrine adenomas) [18]. Although cimetidine effectively promotes ulcer healing and relieves symptoms associated with acid-related disorders, prolonged use has been associated with adverse effects, drug interactions, recurrence of ulceration following treatment discontinuation, and limited protection against oxidative and inflammatory components of NSAID-induced gastric injury [19]. These limitations have stimulated the search for alternative therapeutic agents capable of enhancing endogenous mucosal defense mechanisms.

Cysteine is an Amino acid and also a precursor of Hydrogen-sulphide [20]. N-Acetyl-L-cysteine (NAC), a synthetic derivative of the sulfur-containing amino acid L-cysteine, has attracted considerable research interest because of its potent antioxidant, anti-inflammatory, and cytoprotective properties [21]. N-acetyl-L-cysteine (NAC) serves as a precursor for intracellular glutathione (GSH) synthesis, replenishing cellular GSH stores, enhancing endogenous antioxidant defenses, and mitigating oxidative stress [22,23]. In addition, NAC possesses free radical-scavenging activity, preserves sulfhydryl groups, improves nitric oxide bioavailability, enhances gastric microcirculation, and attenuates inflammatory responses [21]. Recent studies have demonstrated that NAC protects various tissues against oxidative damage and may reduce the severity of NSAID-induced gastric mucosal injury through multiple complementary mechanisms [24,25].

Therefore, given that the precise mechanisms by which N-acetyl-L-cysteine confers protection against NSAID-induced gastric ulceration remain incompletely understood, the present study was designed to investigate its gastroprotective mechanisms, with particular emphasis on its effects on gastric histopathology and selected hematological parameters in an indomethacin-induced gastric ulcer model.

MATERIALS AND METHODS

Experimental animals

Thirty (35) rats weighing (120-150g) each were purchased from *Mctemmy concepts laboratory animals for research and a disease-free animal farm in Osogbo, Osun state, Nigeria*. The animals were kept in polypropylene plastic cages and maintained at normal and standard laboratory conditions of temperature ($28 \pm 2^{\circ}\text{C}$) and relative humidity ($46 \pm 6\%$) with 12-hour light-dark cycle and adequate ventilation for two weeks to allow for acclimatization to their new environment. The rats were weighed to ensure that none of them was outside their initial weight of 120-150g was lost. The entry point weight range was chosen to ensure that all the rats were matured enough for the laboratory experiment. The rats were fed with commercially available standard rat feed (Ladokun feeds Nig. Ltd) purchased from a commercial branch depot in Ondo city, Ondo state. They were given water ad libitum during the period of acclimatization. They were cared for and maintained in the animal facility in accordance with regulations, guidelines and policies governing the use of animals in research as described in public health service policy on human care and use of laboratory animals and approved by the University of

Medical Sciences Animal Research Ethics Committee (UAREC) with approval number/ethical consent code **UNIMED-AREC/Apv/2022/011**.

DRUGS AND REAGENTS

All chemicals used were of analytical grade.

Cimetidine and Indomethacin were purchased from (*well-fast pharmaceuticals*), Ibadan, Nigeria.

L-cysteine was purchased from *Solgar, Inc., 600 Willow Tree Road, Leonia, NJ 07605, U.S.A.*

TREATMENT

The animals to be used for the experiment were pre-treated with L- cysteine and cimetidine for seven (7) days. Rats were fasted, but given access to water, for 24 hours before the experimental procedure.

Group I: Rats served as control received distilled water orally for seven (7) days before administration of indomethacin (40 mg/kg) [26] on the last day (ulcer control).

Group II: Rats received cimetidine orally for seven (7) days (50 mg/kg) [27] and indomethacin orally (40 mg/kg) on the last day.

Group III, IV, V: Rats received NAC at doses of 100, 300, and 500 mg/kg, respectively [28], for seven (7) days orally before indomethacin orally (40 mg/kg) on the last day. Indomethacin, cimetidine, and NAC were dissolved in distilled water and administered orally once daily (once for indomethacin and for seven (7) days for cimetidine and NAC) using a gastric gavage needle.

Route of Administration

All treatments were given by oral route administration, and the pre-treatment lasted for seven (7) days. L- cysteine was dissolved in distilled water after measuring the different doses. Cimetidine was also ground into a fine powder and dissolved in distilled water. All treatment drugs were freshly prepared each day.

INDUCTION OF ULCER

Induction of gastric ulcer was done in the animals using Indomethacin. Following seven (7) days of pretreatment, the animals were fasted for 24 hours, after which indomethacin was administered orally at 40 mg/kg.

ORGAN COLLECTION AND PREPARATION OF TISSUE FOR BIOCHEMICAL ASSAYS.

The animals were sacrificed by cervical dislocation after 4 hours of ulcer induction. The stomachs were removed and cut open for macroscopic examination. They were then opened along the greater curvature, rinsed in ice-cold phosphate-buffered saline, and ulcer scoring was determined. Afterward, they were

blotted and weighed. The stomachs were then minced with scissors into sections; a section was stored in iced-cold 0.1M phosphate buffer, pH 7.4, and homogenized using a homogenizer. The resulting supernatant-post mitochondrial fraction (PMF) was collected and processed for biochemical estimation. The second section was fixed in 10 buffered formalin for histological assessment.

DETERMINATION OF GASTRIC MUCUS SECRETION (Corne, 1974; Oluwole et al., 2007)

The mucus of the gastric wall was determined by the method of Corne *et al.*, (1974) as described by modified by Oluwole *et al.*, (2007). The whole stomach was weighed and transferred into a solution of 0.1% w/v Alcian blue in 0.16 M sucrose solution buffered with 0.05 M Sodium acetate at pH 5.8. The tissue was stained for two hours after which the excess dye was rinsed by two successive rinses with 0.25 M sucrose, for fifteen minutes and subsequently for forty-five minutes. The dye complexed with the gastric mucus was then extracted with 10 ml of 0.5 M $MgCl_2$ and shaken intermittently for one minute at ten minutes' intervals for two hours. Exactly four millilitres (4 ml) of the extract was shaken with equal volume of diethylether. The emulsion obtained was centrifuged at 3600 rpm for 10 minutes and the absorbance of the aqueous layer was recorded at 620 nm. The quality of the dye extracted per gram of wet glandular tissue was then calculated.

DETERMINATION OF SUPEROXIDE DISMUTASE (SOD) ACTIVITY (Beyer Jr & Fridovich, 1987)

Protocol: 1 ml of sample was diluted in 9 ml of distilled water to make a 1 in 10 dilution. An aliquot of 0.2 ml of the diluted sample was added to 2.5 ml of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction started by the addition of 0.3 ml of freshly prepared 0.3 Mm adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5 ml buffer, 0.3 ml of substrate (adrenaline) and 0.2 ml of water. The increase in absorbance at 480 nm was monitored every 30 seconds for 150 seconds.

$$\text{Calculation: Increase in absorbance per minute} = \frac{A_3 - A_0}{2.5}$$

Where A_0 = absorbance after 30 seconds

A_3 = absorbance after 150 seconds

$$\% \text{ Inhibition} = 100 - \frac{\text{Increase in absorbance for substrate} \times 100}{\text{Increase in absorbance for blank}}$$

1 unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline

DETERMINATION OF NITRIC OXIDE ACTIVITY (Moshage et al., 1995)

Nitrite was determined as an oxidation product and indicator of NO synthesis as described by Moshage et al., 1995. This method is based on the addition of Griess reagent, which converts nitrite into a deep purple azo chromophore. The colour intensity was measured using a UV-visible spectrophotometer. Nitric oxide level was expressed as mol/g tissue.

DETERMINATION OF GASTRIC BLOOD FLOW

Gastric blood flow was measured as a component of abdominal aortic blood flow. Abdominal aortic blood flow was measured by placing an ultrasonic Doppler flow probe (Transonic 11RB) around the abdominal aorta between the diaphragm and the celiac artery. Flow rates were obtained with the Transonic T206 Blood Flow Meter (Transonic Instrument, Ithaca, NY).

Animals were fasted, but not deprived of water, for 24 hours before the onset of the experiment. 1 hour before ulcer induction, the test substance was administered to their respective group, after which indomethacin was given to induce ulcer in all groups except group 1 (control group). Animals were anesthetized with ketamine (1ml/kg) intraperitoneally. A midline laparotomy was performed to expose the abdominal aorta for the placement of the probe. The intestine of the rats was deflected to the right to expose the abdominal aorta. Adjacent fats were removed for proper acoustical coupling. The recorded blood flow was expressed in ml/min.

DETERMINATION OF SULFHYDRYL CONTENT

Aliquots of the homogenate were used to assay for the sulfhydryl protein concentration

DETERMINATION OF BLOOD CELL PARAMETERS (PCV, RBC, WBC, AND HAEMOGLOBIN)

An automated blood cell counter was used for complete blood count (CBC) measurements. All samples were run in duplicate, and the mean value was used for statistical analysis.

STATISTICAL ANALYSIS

Data were stated as Mean $\pm$ SEM. Statistical evaluation was made with GraphPad Prism version 9.0. The variations across groups were done with analysis of variance (ANOVA), followed by Turkey's multiple comparisons test for post-hoc comparison of group means. Brown-Forsy test was used for non-parametric variables to determine the differences across groups, where $p \leq 0.05$ was considered significant.

Data Availability Statement:

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

RESULTS

FIGURE 1: EFFECT OF CYSTEINE AND CIMETIDINE PRE-TREATMENT ON NITRIC OXIDE ACTIVITY IN INDOMETHACIN-INDUCED ULCERATION IN RATS

There is a significant difference in the Nitric Oxide (NO) activity between all cysteine-treated groups and also cimetidine alone (Cysteine 100 mg/kg: 8.41 ± 0.06 ; Cysteine 300 mg/kg: 10.37 ± 0.18 $\mu\text{mol/g}$; Cysteine 500 mg/kg: 12.23 ± 0.44 $\mu\text{mol/g}$; Cimetidine alone 50 mg/kg: 7.997 ± 0.24 $\mu\text{mol/g}$). These groups showed significantly higher nitric oxide activity (especially the group treated with 500mg/kg cysteine) compared with the ulcer control group (7.852 ± 0.09062 $\mu\text{mol/g}$).

FIGURE 2: EFFECT OF CYSTEINE AND CIMETIDINE PRE-TREATMENT ON GASTRIC BLOOD FLOW IN INDOMETHACIN INDUCED ULCERATION IN RATS

The gastric blood flow was significantly high in all the treated groups but especially the groups treated with cysteine 300mg/kg and 500mg/kg as they show more significant increase compare to the ulcer control, (Cysteine 100 mg/kg: 1.87 ± 0.39 ml/min; Cysteine 300 mg/kg: 2.11 ± 0.6 ml/min; Cysteine 500 mg/kg: 3.2 ± 0.09 ml/min; Cimetidine alone 50 mg/kg: 1.20 ± 0.15 ml/min) ulcer control group (1.12 ± 0.03 ml/min). This result is illustrated in Figure 2 below.

FIGURE 3: EFFECT OF CYSTEINE AND CIMETIDINE PRE-TREATMENT ON SULFHYDRYL GROUP IN INDOMETHACIN-INDUCED ULCERATION IN RATS

There was a significant increase in sulfhydryl level in high (500 mg/kg) and medium (300 mg/kg) cysteine-treated groups and cimetidine treated group alone (Cysteine 500 mg/kg: $0.00 \pm 3.33 \times 10^{-5}$ M; Cysteine 300 mg/kg: $0.00 \pm 3.33 \times 10^{-5}$ M; Cimetidine alone: $0.00 \pm 3.33 \times 10^{-5}$ M) when compared with the ulcer control ($9.66 \times 10^{-5} \pm 3.33 \times 10^{-6}$ M). However, the sulfhydryl level of the low cysteine-treated group (100 mg/kg) (Cysteine low $0.00 \pm 3.33 \times 10^{-5}$ M) shows no significant difference compared to the ulcerated control. This result is illustrated in Figure 3

FIGURE 4: EFFECT OF CYSTEINE AND CIMETIDINE PRE-TREATMENT ON SUPEROXIDE DISMUTASE (SOD) IN INDOMETHACIN INDUCED ULCERATION IN RATS

Superoxide dismutase activity increases in the groups treated with cysteine low, medium and high doses (Cysteine 100 mg/kg: 10.69 ± 0.10 $\mu\text{g/mg protein}$; Cysteine 300 mg/kg: 10.12 ± 0.29 $\mu\text{g/mg protein}$; Cysteine 500 mg/kg: 14.21 ± 0.51 $\mu\text{g/mg protein}$) but shows more significance in the high dose. It also shows significant difference in the group treated with cimetidine alone (11.02 ± 0.23 $\mu\text{g/mg protein}$). This result is represented in figure 6 below

FIGURE 5: EFFECT OF CYSTEINE AND CIMETIDINE PRE-TREATMENT ON GASTRIC MUCUS SECRETION

Mucus secretion in pre-treated rats increases and it is significant in difference in the groups treated with cysteine high, medium and low doses (Cysteine 100 mg/kg: 0.919 ± 0.001 mg/g; Cysteine 300 mg/kg: 0.941 ± 0.001 mg/g; Cysteine 500 mg/kg: 0.998 ± 0.001 mg/g), it shows less significant difference in the group treated with cimetidine alone 50 mg/kg (0.835 ± 0.004 mg/g) when compared with the ulcer control (0.815 ± 0.013 mg/g). This result is represented in figure 5 below

TABLE 1: EFFECT OF CYSTEINE AND CIMETIDINE PRE-TREATMENT ON HEMATOLOGICAL PARAMETERS IN INDOMETHACIN-INDUCED ULCER MODEL

GROUPS	PVC (%)	WBC COUNTS ($10^9/L$)	RBC COUNTS (10^6 Cells/uL)	HEMOGLOBIN CONC (g/dL)
Control	44.60 ± 0.74	3025 ± 59.51	7.67 ± 0.19	15.85 ± 0.40
Cysteine 100 mg/kg	$50.60 \pm 0.24^{**}$	$3638 \pm 143.40^*$	$8.55 \pm 0.15^*$	$17.38 \pm 0.21^*$
Cysteine 300 mg/kg	$51.60 \pm 0.24^{**}$	$4086 \pm 51.54^{***}$	$9.23 \pm 0.16^{**}$	$18.9 \pm 0.26^{**}$
Cysteine 500 mg/kg	$52.40 \pm 0.24^{**}$	$3963 \pm 114.30^{**}$	$10.53 \pm 0.09^{***}$	$20.33 \pm 0.29^{**}$
Cimetidine only 50 mg/kg	$48.60 \pm 0.24^*$	$4050 \pm 165.80^{***}$	$8.89 \pm 0.20^*$	$16.38 \pm 0.04^*$

* Significant at $p < 0.05$ when compared with ulcer control

** More significant at $p < 0.005$ when compared with ulcer control

*** Most significant at $p < 0.0005$ when compared with ulcer control

Table 1 shows that:

There is a significant difference in the Packed Cell Volume in different doses of cysteine-treated groups, as well as cimetidine alone (Cysteine 100 mg/kg: $50.60 \pm 0.24\%$; Cysteine 300 mg/kg: $51.60 \pm 0.24\%$; Cysteine 500 mg/kg: $52.40 \pm 0.24\%$; Cimetidine alone (50 mg/kg): $48.60 \pm 0.24\%$) when compared to the ulcer control group ($44.60 \pm 0.74\%$).

There is a significant difference in the white blood cell count in all treated groups (Cysteine at different doses and Cimetidine alone) (Cysteine 100 mg/kg: 3638 ± 143.40 million cu/mm; Cysteine 300 mg/kg: 4086 ± 51.54 million cu/mm; Cysteine 500 mg/kg: 3963 ± 114.30 million cu/mm; Cimetidine alone (50 mg/kg): 4050 ± 165.80 million cu/mm) when compared to the ulcer control group (3025 ± 59.51 million cu/mm).

The red blood cell count shows significant difference in all treated groups (Cysteine at different doses and Cimetidine alone) (Cysteine 100 mg/kg: 8.55 ± 0.15 million cu/mm; Cysteine 300 mg/kg: 9.23 ± 0.16

million cu/mm; Cysteine 500 mg/kg: 10.53 ± 0.09 million cu/mm; Cimetidine alone 50 mg/kg: 8.89 ± 0.20 million cu/mm) when compared to the ulcer control group (7.67 ± 0.19 million cu/mm).

The haemoglobin concentration in the treated groups of different doses and cimetidine alone shows a significant difference when compared to the ulcer control group (Cysteine 100 mg/kg: 17.38 ± 0.21 g/dl; Cysteine 300 mg/kg: 18.9 ± 0.26 g/dl; Cysteine 500 mg/kg: 20.33 ± 0.29 g/dl; Cimetidine 50 mg/kg alone: 16.38 ± 0.04 g/dl), ulcer control group (15.85 ± 0.40 g/dl).

DISCUSSION

Non-steroidal anti-inflammatory drugs (NSAIDs) remain one of the leading causes of gastric ulceration owing to their ability to disrupt the balance between mucosal defensive mechanisms and aggressive luminal factors [5,3]. Indomethacin, a potent non-selective cyclooxygenase (COX) inhibitor, induces gastric injury primarily through suppression of prostaglandin synthesis, resulting in diminished mucus and bicarbonate secretion, impaired mucosal blood flow, reduced epithelial restitution, and increased gastric acid-mediated injury [29,10]. Beyond prostaglandin depletion, indomethacin promotes excessive generation of reactive oxygen species (ROS), mitochondrial dysfunction, neutrophil infiltration, and microvascular ischemia, all of which synergistically contribute to epithelial disruption and ulcer formation [30].

The present study demonstrates that N-acetylcysteine (NAC) confers marked protection against indomethacin-induced gastric injury by preserving gastric secretory function, strengthening antioxidant defenses, and maintaining hematological homeostasis. The gastroprotective effect of NAC is likely mediated through multiple interconnected mechanisms rather than a single pharmacological action [21].

One of the earliest consequences of indomethacin administration observed in this study was increased gastric acidity accompanied by a reduction in gastric pH and depletion of gastric mucus. Increased hydrogen ion concentration within the gastric lumen accelerates mucosal injury by facilitating back-diffusion of acid into damaged epithelial cells, disrupting intracellular pH homeostasis, denaturing cellular proteins, and promoting cell necrosis [31, 32]. Simultaneously, prostaglandin depletion reduces mucus and bicarbonate secretion, exposing the gastric epithelium directly to luminal acid and pepsin [33]. The significant increase in gastric juice volume and total acidity observed in ulcerated animals, therefore, reflects impairment of normal gastric mucosal defense mechanisms, as it correlates with a recent study [34].

Pretreatment with NAC significantly elevated gastric pH while simultaneously increasing gastric mucus content, particularly at higher doses, indicating restoration of the gastric mucosal barrier. Although NAC possesses mucolytic activity in the respiratory tract through reduction of disulfide bonds within mucus glycoproteins, its actions in the gastrointestinal tract appear fundamentally different [35]. Under conditions of oxidative stress, NAC preserves mucus integrity indirectly by limiting ROS-mediated degradation of mucin glycoproteins, maintaining epithelial cell viability, and restoring prostaglandin-dependent mucosal secretory activity [36]. Consequently, the observed increase in gastric mucus

following NAC treatment most likely reflects preservation of mucus synthesis and secretion rather than direct modification of mucus flow.

Oxidative stress constitutes a central mechanism underlying NSAID-induced gastric injury. Excessive production of superoxide anions, hydrogen peroxide, hydroxyl radicals, and lipid peroxidation products overwhelms endogenous antioxidant defenses, resulting in membrane lipid oxidation, mitochondrial dysfunction, DNA damage, and activation of apoptotic signaling pathways [37]. Superoxide dismutase (SOD) represents the first enzymatic line of antioxidant defense by catalyzing the dismutation of superoxide radicals into hydrogen peroxide, which is subsequently detoxified by catalase and glutathione peroxidase. Consistent with previous reports, indomethacin significantly reduced gastric SOD activity, indicating severe oxidative stress within gastric tissues [38, 39].

Also in this study, it was observed that both H₂S donor (L-cysteine) and H₂S precursor (L-cysteine) decreased indomethacin-induced gastropathy. These findings are consistent with the hypothesis that the gastroprotective effects of L-cysteine are correlated to increase H₂S synthesis [40, 41], which could be accountable for the significant increase in the sulfhydryl concentration in all the cysteine-treated groups and the group treated with cimetidine alone.

H₂S has long been recognized as an industrial pollutant and has also been increasingly identified as an important mediator of many physiological processes [42]. H₂S has been proven to be a neuromodulator, as well as endogenous regulator of acute inflammation and pain [43, 44]. It was also observed H₂S being a precursor of NAC demonstrated that it contributes to the maintenance of gastric mucosal integrity in damaged cells caused by nonsteroidal anti-inflammatory drugs [45].

Administration of NAC significantly restored SOD activity in a dose-dependent manner, with the greatest effect observed at 500 mg/kg. This finding is mechanistically relevant because NAC serves as an exogenous source of cysteine, the rate-limiting substrate for glutathione (GSH) biosynthesis [46]. Restoration of intracellular GSH enhances cellular redox buffering capacity, facilitates detoxification of hydrogen peroxide through glutathione peroxidase, preserves mitochondrial function, and prevents oxidative inactivation of endogenous antioxidant enzymes [47, 48]. Furthermore, the free sulfhydryl (-SH) group of NAC directly scavenges highly reactive oxygen and nitrogen species, thereby interrupting lipid peroxidation chain reactions and protecting cellular membranes from oxidative injury [49]. The improvement in SOD activity, therefore, likely reflects both preservation of enzyme integrity and attenuation of oxidative burden within the gastric mucosa, as reported in recent studies [23].

Another important mechanism that may contribute to the gastroprotective action of NAC is preservation of nitric oxide (NO) bioavailability. Oxidative stress rapidly inactivates NO through reaction with superoxide to form peroxynitrite, thereby impairing endothelium-dependent vasodilation [50]. By reducing superoxide accumulation and replenishing intracellular thiol pools, NAC may increase NO bioavailability, leading to improved gastric microcirculation [24]. Adequate mucosal perfusion is essential for the delivery of oxygen and nutrients, the removal of toxic metabolites, the maintenance of epithelial energy

metabolism, and the acceleration of tissue repair following injury. Thus, the beneficial effects of NAC on gastric blood flow observed in this study are likely secondary to its antioxidant properties and preservation of endothelial NO signaling.

Beyond its antioxidant effects, NAC possesses important anti-inflammatory properties that may further explain its gastroprotective efficacy. ROS generated following indomethacin administration activate redox-sensitive transcription factors, particularly nuclear factor-kappa B (NF- κ B), leading to increased expression of pro-inflammatory cytokines such as TNF- α , IL-1 β , and IL-6, as well as enhanced neutrophil recruitment [51]. Activated neutrophils amplify tissue injury through the release of proteolytic enzymes, myeloperoxidase, and additional ROS, creating a vicious cycle of inflammation and oxidative damage [52]. By suppressing oxidative stress, NAC indirectly limits activation of these inflammatory pathways, thereby reducing secondary tissue destruction and promoting mucosal healing.

The hematological findings further support the protective effects of NAC. Indomethacin-induced gastric ulceration may result in mucosal hemorrhage and systemic inflammatory responses that alter erythrocyte indices and leukocyte counts [53, 54]. Pretreatment with NAC significantly improved packed cell volume, hemoglobin concentration, erythrocyte count, and leukocyte profile compared with ulcer controls. Preservation of red blood cell indices may reflect reduced gastric bleeding resulting from enhanced mucosal integrity, while normalization of leukocyte counts is consistent with attenuation of systemic inflammatory activation [55]. The improved oxygen-carrying capacity, associated with preserved erythrocyte parameters, may also contribute to enhanced tissue oxygenation and mucosal repair.

Conclusion

Collectively, this study suggests that NAC exerts gastroprotection through multiple complementary mechanisms involving: restoration of redox homeostasis, preservation of endogenous antioxidant defenses, maintenance of nitric oxide bioavailability, enhancement of gastric microcirculation, stabilization of the mucus barrier, suppression of oxidative stress-induced inflammation and improvement of hematological status. The dose-dependent responses observed in the present study further indicate that the antioxidant and cytoprotective effects of NAC become increasingly effective with higher therapeutic doses. These integrated mechanisms likely underlie the substantial attenuation of indomethacin-induced gastric mucosal injury observed in this experimental model. Thus, it has the potential for further development as a promising alternative for the treatment of ulcer.

Recommendation

It is suggested that further studies should be done on the particular gastro-protective effective dose of cysteine alone, and in combination with cimetidine.

Contribution to Knowledge

L-cysteine pre-treatment prevented gastric ulceration induced by indomethacin, somewhat better than cimetidine, especially if used as combination therapy in the treatment of gastric ulcer.

Declarations

Conflict of Interest Statement

No conflict-of-interest statement

Funding Statement

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Author Contribution

O.V: Conceptualization, Methodology, Funding, Supervision, Analysis, Writting of original manuscript, Manuscript review
A.A: Funding, Writting of original manuscript, Manuscript review
B.C: Writting of original manuscript, Manuscript review

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Data Availability

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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Figures

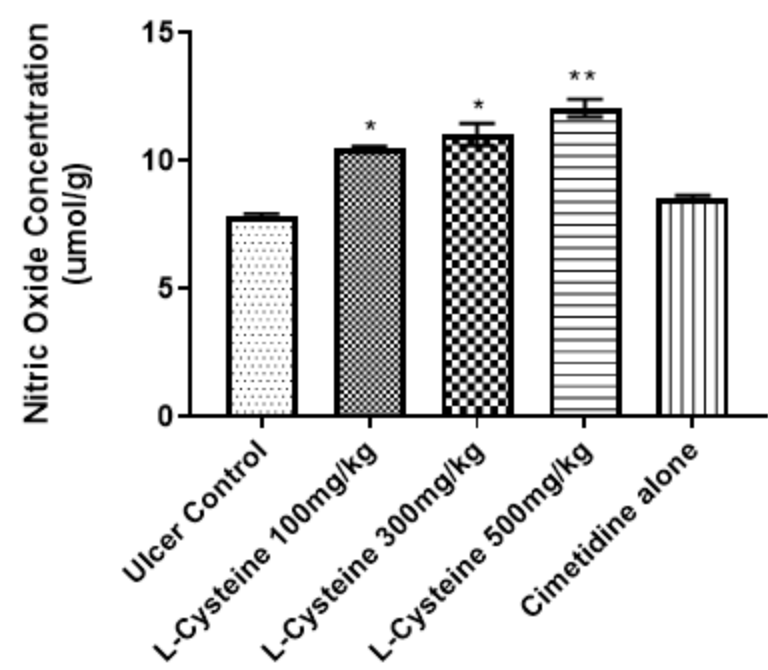


Figure 1

Effect of Cysteine on Nitric Oxide activity in Indomethacin-induced ulceration

*Significant at $p<0.05$ when compared with ulcer control.

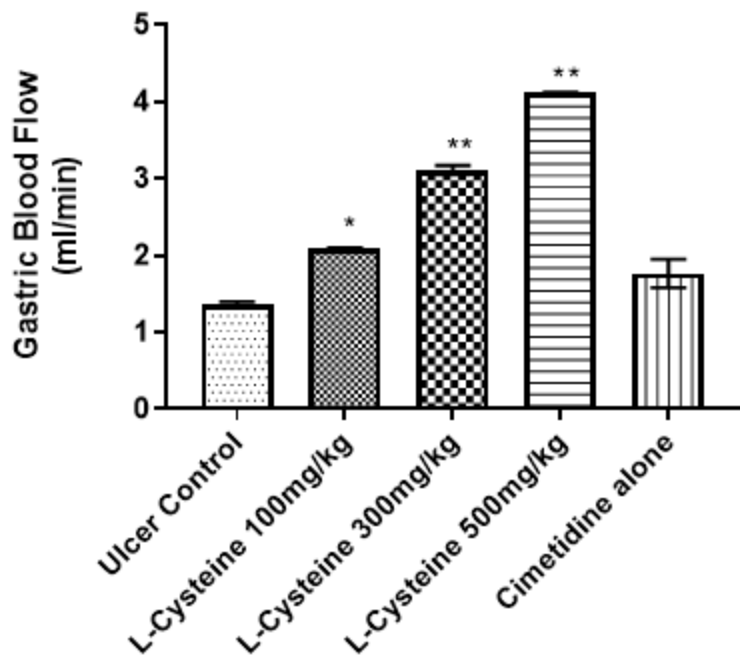


Figure 2

Effect of Cysteine on Gastric Blood Flow in Indomethacin induced ulceration

*Significant at $p < 0.05$ when compared with ulcer control

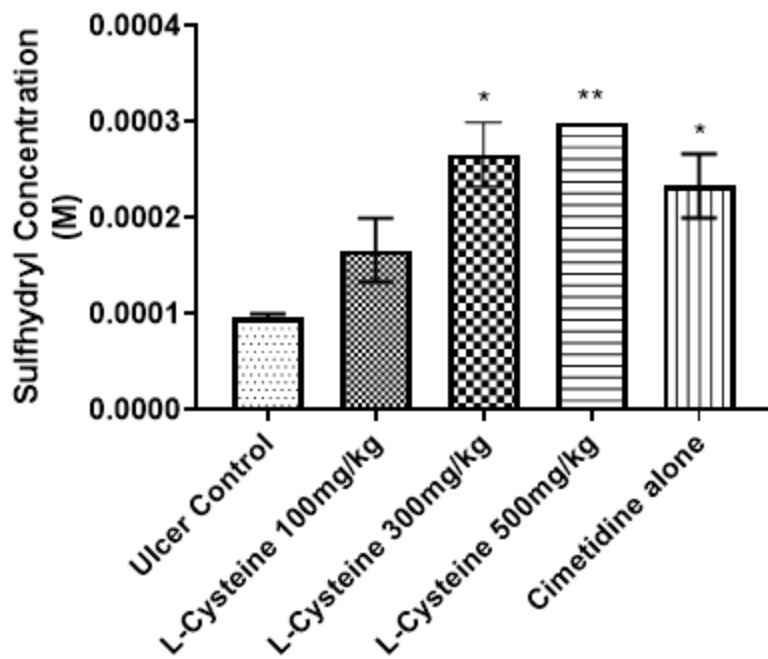


Figure 3

Effect of Cysteine on sulfhydryl content in Indomethacin induced ulceration in rats

*Significant at $p<0.05$ when compared with ulcer control

** Very significant at $p<0.005$ when compared with ulcer control

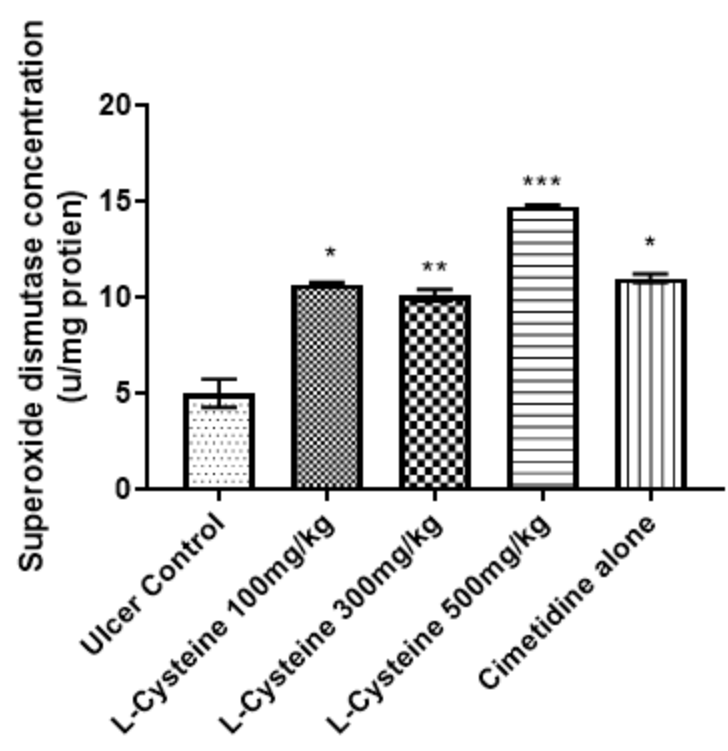


Figure 4

Effect of Cysteine on Superoxide Dismutase (SOD) in Indomethacin induced ulceration in rats

*Significant at $p<0.05$ when compared with ulcer control

** Very significant at $p<0.005$ when compared with ulcer control

*** Mostly significant at $p<0.0005$ when compared with ulcer control

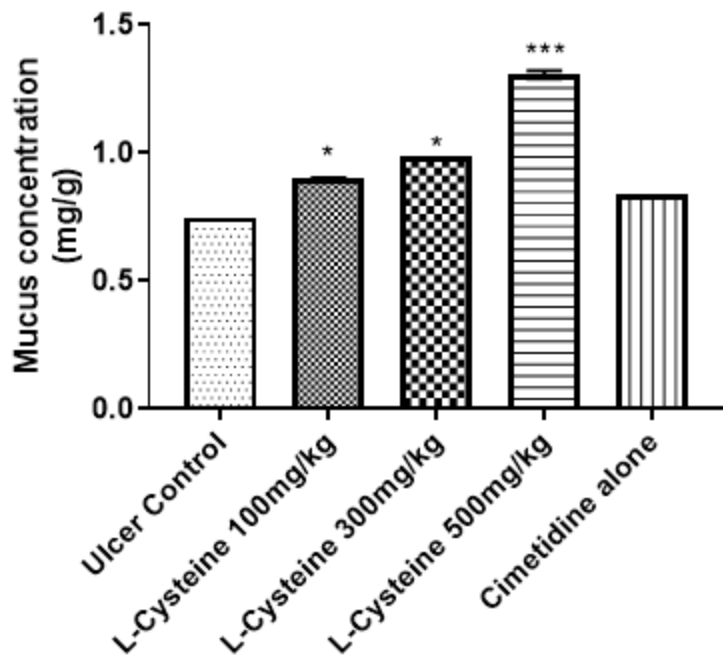


Figure 5

Effect of Cysteine on mucus secretion in Indomethacin induced ulceration in rats.

*Significant at $p < 0.05$ when compared with ulcer control

** Very significant at $p < 0.005$ when compared with ulcer control

*** Highly significant at $p < 0.0005$ when compared with the control