RESEARCH PAPER

Evaluation of the anti-ulcer activity of L-arginine against indomethacin-induced gastric ulcer in rats

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Abstract

The widespread clinical utility of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) is severely limited by their propensity to induce gastric mucosal injury through the suppression of cytoprotective prostaglandins and the generation of oxidative stress. The present study evaluated the gastroprotective and anti-ulcerogenic efficacy of L-Arginine, a physiological precursor of nitric oxide (NO), against Indomethacin-induced gastric ulcers in Wistar rats. Experimental animals were divided into five groups and pretreated with varying doses of L-Arginine or the standard proton pump inhibitor, Omeprazole, prior to a single oral challenge with Indomethacin (30 mg/kg). The assessment included macroscopic determination of the Ulcer Index, biochemical analysis of gastric juice (pH, free and total acidity), and evaluation of mucosal defense factors (mucin and pepsin content), substantiated by histopathological examination. Results demonstrated that Indomethacin administration triggered severe hemorrhagic lesions, hyperacidity, and significant depletion of the mucus barrier. Conversely, pretreatment with L-Arginine significantly ($P<0.01$) and dose-dependently attenuated gastric mucosal damage, reducing the Ulcer Index and suppressing aggressive proteolytic pepsin activity. Furthermore, L-Arginine therapy effectively restored gastric pH towards neutrality and preserved glandular mucin content, exhibiting efficacy comparable to the standard drug Omeprazole. Histological analysis corroborated these findings, revealing preserved mucosal architecture with reduced submucosal edema and minimal leukocyte infiltration. These findings suggest that L-Arginine exerts a potent cytoprotective effect, likely mediated by the modulation of the nitric oxide pathway, which enhances mucosal perfusion and fortifies the epithelial barrier against NSAID-induced gastropathy.

Keywords: L-Arginine; Indomethacin; Gastric Ulcer; Nitric Oxide; Mucin; Cytoprotection.

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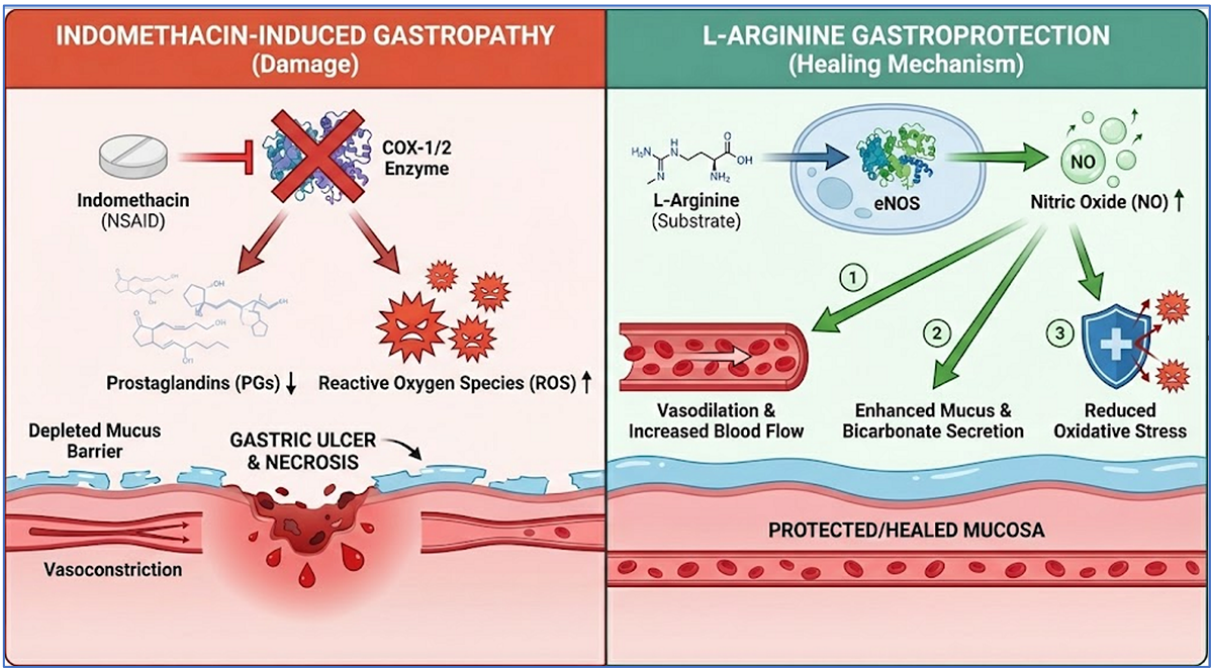

GRAPHICAL ABSTRACT

1. Introduction

The gastrointestinal tract is a complex network of hollow organs, among which the stomach plays a pivotal role in the digestion of dietary intake. Despite its resilience against noxious stimuli such as hydrochloric acid, refluxed bile salts, and alcohol, the stomach's integrity relies on a delicate physiological equilibrium (Lanas & Chan, 2017). Peptic ulcer disease (PUD), characterized by a breach in the lining of the gastrointestinal mucosa that extends into the submucosa, represents a significant clinical challenge where this equilibrium is disrupted (Rosenstock & Jørgensen, 1995). PUD remains one of the most prevalent gastrointestinal disorders globally, affecting approximately 10% of the world's population (Lanas & Chan, 2017). In the United States alone, the lifetime risk of developing a peptic ulcer is estimated at 10% for men and 4% for women, with nearly 350,000 new cases diagnosed annually (Kurata et al., 1992). The etiology of this condition is multifactorial, driven by an imbalance where aggressive factors such as acid, pepsin, and reactive oxygen species (ROS) overwhelm the mucosal defense mechanisms (Graham, 2014). Among the exogenous aggressive factors, the widespread consumption of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) is a dominant cause of gastric mucosal injury (Wallace et al., 2000a; Wallace et al., 2000b). NSAIDs are extensively employed for the management of pain, inflammation, and chronic conditions such as rheumatoid arthritis and osteoarthritis, with over 35 million individuals consuming them daily worldwide (Lanas & Chan, 2017). While these agents are clinically indispensable, they present a "double-edged sword" therapeutic profile. NSAIDs are responsible for inducing gastroduodenal ulcers in approximately 25% of chronic users, often leading to severe complications such as hemorrhage and perforation (Wallace et al., 2000a). The pathophysiology of indomethacin-induced gastric ulceration is complex and primarily mediated through the inhibition of the cyclooxygenase (COX) enzyme system. The primary pharmacological action of NSAIDs involves the competitive antagonism of COX, thereby blocking the conversion of arachidonic acid into prostaglandins (PGs) (Wallace et al., 2000a; Wallace et al., 2000b). Prostaglandins, particularly those derived from the constitutive COX-1 isoform, are essential for maintaining gastrointestinal integrity; they regulate mucosal blood flow, stimulate bicarbonate and mucus secretion, and maintain cellular homeostasis (Lanas & Chan, 2017). Indomethacin, by non-selectively inhibiting COX enzymes, depletes these cytoprotective prostaglandins, rendering the mucosa susceptible to injury (Wallace et al., 2000a). Beyond the suppression of prostaglandins, oxidative stress plays a fundamental role in the pathogenesis of gastric lesions. The accumulation of reactive oxygen species (ROS) such as superoxide anions, hydrogen peroxide, and hydroxyl radicals leads to lipid peroxidation and cellular damage (Bandyopadhyay et al., 2002). The overwhelming body of evidence suggests that oxidative stress is a primary cause of tissue necrosis in gastric ulcers (Wallach, 1997). Furthermore, NSAIDs induce microvascular injury by increasing the expression of adhesion molecules, which promotes neutrophil adherence to the vascular endothelium (Wihastuti et al., 2007). This neutrophil accumulation results in the release of proteases and free radicals that obstruct capillary blood flow, leading to ischemia and subsequent mucosal necrosis (Wallace et al., 2000b). Currently, the management of peptic ulcers relies heavily on synthetic pharmaceuticals, including proton pump inhibitors (PPIs) like omeprazole, H2-receptor antagonists, and antacids (Lanas & Chan, 2017). While

effective in neutralizing acid or inhibiting its secretion, these conventional therapies are associated with significant limitations. Long-term use of synthetic anti-ulcer drugs can lead to adverse effects, including hypercalcemia, nutrient malabsorption, and potential liver toxicity (Lanas & Chan, 2017). Moreover, the high cost of these regimens and the incidence of relapse upon withdrawal pose challenges, particularly in developing regions (Rosenstock & Jørgensen, 1995). Consequently, there is a growing interest in identifying endogenous or natural compounds that can offer gastroprotection with a superior safety profile. This has led researchers to investigate molecules that can bolster the stomach's intrinsic defensive barriers rather than merely suppressing acid production (Asaad & Mostafa, 2022; Dejban et al., 2020). In this context, L-arginine emerges as a promising therapeutic candidate. While previous studies have explored endogenous mediators and metabolic pathways involved in cytoprotection and tissue preservation, L-arginine offers a distinct mechanistic advantage through the nitric oxide (NO) pathway (Dejban et al., 2020). L-arginine is the physiological precursor to nitric oxide, a gaseous mediator now recognized as a critical regulator of gastric mucosal defense (Wallace et al., 2000a). Recent research has increasingly emphasized NO's role in maintaining mucosal integrity in a manner analogous to prostaglandins (Lanas & Chan, 2017). The rationale for evaluating L-arginine lies in its capacity to modulate the nitric oxide synthase (NOS) pathway. Nitric oxide functions as an endogenous vasodilator, playing a vital role in regulating mucosal blood flow and reducing epithelial permeability, thereby increasing the mucosa's resistance to ulceration (Wallace et al., 2000b). Crucially, NO has been shown to inhibit leukocyte adhesion to the vascular endothelium, directly counteracting the microvascular injury mechanisms initiated by NSAIDs such as indomethacin (Wihastuti et al., 2007; Dejban et al., 2020). Furthermore, NO influences mucus and bicarbonate secretion, reinforcing the physical barrier against acid–pepsin digestion (Lanas & Chan, 2017). Evidence also indicates that suppression of NO production markedly increases gastric vulnerability, whereas administration of NO donors reduces inflammation and accelerates ulcer healing (Bandyopadhyay et al., 2002). Despite the established understanding of NO's gastroprotective role, there remains a need to systematically evaluate the therapeutic efficacy of its direct precursor, L-arginine, specifically against the severe oxidative and ischemic injury induced by indomethacin (Dejban et al., 2020). Unlike synthetic agents that primarily suppress acid secretion, L-arginine supplementation is proposed to restore defensive homeostasis by enhancing mucosal perfusion and attenuating oxidative stress (Asaad & Mostafa, 2022). The present study hypothesizes that L-arginine will mitigate indomethacin-induced gastropathy by reversing vasoconstriction, limiting ROS-mediated damage, and preserving gastric microarchitecture (Bandyopadhyay et al., 2002; Wallace et al., 2000b). By evaluating parameters such as gastric juice volume, pH, mucin content, and histopathological alterations, this investigation seeks to establish L-arginine as a physiologically compatible and potent anti-ulcer agent, potentially bridging the gap between therapeutic efficacy and safety in the management of peptic ulcer disease.

2. Materials and Methods

2.1 Drugs and Chemicals

The test drug, L-Arginine, and the standard drug, Omeprazole, were procured from reputable pharmaceutical suppliers (Tata 1mg/Mankind Pharmaceuticals). Indomethacin was used as the ulcerogenic agent. All other chemicals and reagents used in the study were of analytical grade. The drug suspensions were prepared fresh prior to administration.

2.2 Experimental Animals

Healthy adult Wistar rats of either sex, weighing between 180–220 g, were employed for the present investigation. The animals were housed in standard polypropylene cages under controlled environmental conditions (25 ± 2°C, 12-hour light/dark cycle) with *ad libitum* access to standard laboratory food pellets and tap water. The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC). The care and handling of animals were conducted in strict compliance with the guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

2.3 Experimental Design

The animals were randomly divided into five groups, with six animals in each group (n=6). The study followed a treatment protocol where the test drug (L-Arginine) and standard drug (Omeprazole) were administered orally as a pretreatment for a specific duration (e.g., 10 days) prior to ulcer induction. On the day of ulcer induction, animals were fasted overnight (water *ad libitum*), and gastric ulcers were induced using a single dose of Indomethacin (30 mg/kg, p.o.). The grouping and treatment schedule are detailed in Table 1.

Table 1: Experimental Design for Anti-Ulcer Activity

Group	Group Name	Treatment Regimen	Number of Animals
G-I	**Normal Control**	Normal Saline (2 ml/kg, p.o.)	6
G-II	**Disease Control**	Single dose Indomethacin (30 mg/kg, p.o.)	6
G-III	**L-Arginine Low Dose**	L-Arginine (Low Dose, p.o.) + Indomethacin (30 mg/kg)	6

G-IV	**L-Arginine High Dose**	L-Arginine (High Dose, p.o.) + Indomethacin (30 mg/kg)	6
G-V	**Standard Treatment**	Omeprazole (40 mg/kg, p.o.) + Indomethacin (30 mg/kg)	6

2.4 Induction of Gastric Ulcer

Gastric ulcers were induced in the experimental animals (Groups II-V) using indomethacin, following a standard protocol. Prior to the induction of ulcers, the rats were fasted overnight (approximately 18-24 hours) to ensure the stomach was empty, though they were allowed free access to water. On the day of induction, a single dose of indomethacin (30 mg/kg body weight) was administered orally to the rats in the disease control and treatment groups. The drug was suspended in an appropriate vehicle (e.g., 0.5% carboxymethyl cellulose or saline) to ensure uniform delivery. Following the administration of indomethacin, the animals were kept under observation. Twenty-four hours after the ulcerogenic challenge, the animals were sacrificed to evaluate the severity of gastric mucosal injury and biochemical parameters (Wallace et al., 2000).

2.5 Evaluation Parameters

2.5.1 Macroscopic Evaluation (Ulcer Score and Index)

- Immediately after sacrifice, the stomachs were excised, opened along the greater curvature, and rinsed with saline to remove gastric contents. The mucosal surface was examined macroscopically for lesions using a magnifying glass. The severity of mucosal damage was graded using an arbitrary scale as described by Dejban et al. (2020).
- **0:** No ulcer or normal stomach.
- **1:** Superficial mucosal erosion.
- **2:** Deep ulcer or transmural necrosis.
- **3:** Perforated or penetrated ulcer.

The Ulcer Index (UI) was calculated to quantify the extent of damage. As per the protocol, the ulcer index is derived from the relationship between the total mucosal area and the ulcerated area.

2.5.2 Gastric Secretion Analysis

- The gastric juice collected from the stomach was centrifuged to remove solid debris. The volume of the supernatant was measured and expressed as mL/100 g body weight (Asaad & Mostafa, 2022).
- **pH Measurement:** The pH of the gastric juice was determined using a calibrated pH strip or meter.
- **Acidity Estimation:** Total and free acidity were estimated by titrating 1 mL of gastric juice against 0.01 N NaOH.
    - **Free Acidity:** Determined using Topfer's reagent as an indicator; titration continued until the solution turned canary yellow.
    - **Total Acidity:** Determined by adding phenolphthalein to the solution and continuing titration until a permanent pink color was achieved.

Acidity was expressed as meq/L/100g using the formula:

Volume of NaOH × Normality of NaOH × 100 / 0.1 E.q1

2.5.3 Assessment of Mucosal Defense Factors

Gastric Mucin Content: The barrier mucus was estimated using the Alcian Blue binding method. The everted stomachs were soaked in 0.1% Alcian blue 8GX solution (buffered with sodium acetate, pH 5.8) for two hours. Uncomplexed dye was removed by washing in sucrose solution. The dye complexed with the mucus wall was then extracted using 0.5 M $MgCl_2$, and the optical density was measured spectrophotometrically at 605 nm. Mucin content was expressed as µg of Alcian blue per gram of wet tissue (De Araújo et al., 2018; Asaad & Mostafa, 2022).

Pepsin Activity: Pepsin activity in the gastric juice was evaluated using albumin as a substrate. An aliquot of gastric juice (20 µl) was incubated with albumin solution (5 mg/ml) at 37 °C for 10 minutes. The reaction was stopped using 10% trichloroacetic acid (TCA), and the mixture was centrifuged. The supernatant was treated with Folin–Ciocalteu reagent and sodium carbonate, and the absorbance was measured at 660 nm (Asaad & Mostafa, 2022; de Freitas Rocha et al., 2023).

2.5.4 Estimation of Nitric Oxide and Antioxidant Markers

Nitric Oxide (NO) Estimation: Since L-arginine is a precursor to nitric oxide (NO), gastric nitrite levels, a stable metabolite of NO, were estimated using the Griess reaction. The supernatant of the gastric homogenate was mixed with Griess reagent (1% sulfanilamide and 0.1% N-(1-naphthyl)ethylenediamine dihydrochloride). The absorbance was measured at 540 nm to quantify nitric oxide production, indicating the preservation of endothelial function (Dejban et al., 2020; Wallace et al., 2000b).

Antioxidant Enzyme Assays: To assess the reduction in Indomethacin-induced oxidative stress, the gastric tissue homogenate will be analyzed for:

- **Superoxide Dismutase (SOD):** Measured by the inhibition of nitroblue tetrazolium (NBT) reduction.
- **Catalase (CAT):** Determined by monitoring the decomposition of hydrogen peroxide (H_2O) at 240 nm.

- **Lipid Peroxidation (MDA):** Malondialdehyde levels will be measured using the Thiobarbituric Acid (TBA) assay to assess membrane damage.

2.6 Histopathological Examination

2.6.1 Tissue Processing and Staining

Following the macroscopic evaluation, a representative portion of the glandular stomach was excised and immediately fixed in 10% neutral buffered formalin to preserve tissue architecture. After fixation, the tissue samples underwent a standard histological processing protocol, including dehydration in graded series of ethanol, clearing in xylene, and embedding in paraffin wax blocks. Sections of 5 µm thickness were cut using a rotary microtome and mounted on glass slides. The sections were subsequently deparaffinized and stained with hematoxylin and eosin (H&E) (De Araújo et al., 2018; de Freitas Rocha et al., 2023).

2.7 Statistical Analysis

The quantitative data obtained from the experiments were expressed as the Mean ± Standard Error of the Mean (S.E.M.). To determine the statistical significance of the differences between the experimental groups, the data were analyzed using One-way Analysis of Variance (ANOVA). Following the ANOVA, Dunnett's multiple comparison post-hoc test was employed to compare the treatment groups (L-Arginine and Omeprazole) against the Disease Control group. Statistical significance was set at a probability value of $p < 0.05$.

3. Results

3.1 Macroscopic Effect on Gastric Mucosa

The macroscopic examination of the excised stomachs revealed distinct morphological differences across the experimental groups.

- **Normal Control (Group I):** The gastric mucosa of rats in the normal control group appeared healthy, smooth, and intact, showing no evidence of hyperemia or lesions.
- **Disease Control (Group II):** In contrast, the administration of Indomethacin (30 mg/kg) resulted in severe gastric mucosal injury. The stomachs in this group exhibited extensive hemorrhagic lesions, linear ulcers along the greater curvature, and widespread hyperemia. This group demonstrated the maximum Ulcer Score and Ulcer Index, confirming the successful induction of gastropathy.

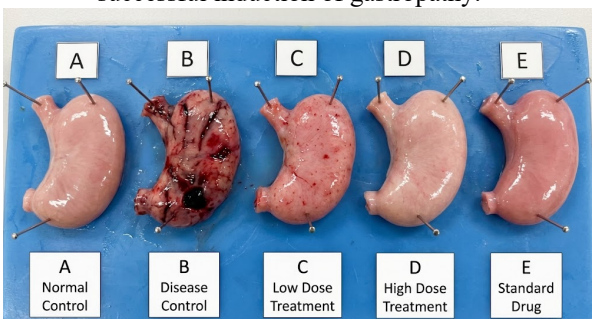

Figure 1: Macroscopic presentation of the gastric mucosa in experimental rats. (A) Normal Control: Shows intact mucosa with no lesions. (B) Disease Control (Indomethacin): Exhibits severe hemorrhagic lesions and necrotic patches (arrows). (C) L-Arginine Low Dose: Shows mild hyperemia with reduced ulceration. (D) L-Arginine High Dose: Shows significant mucosal protection with negligible damage. (E) Standard (Omeprazole): Shows complete protection similar to the normal control.

Effect of L-Arginine Treatment:

Pretreatment with L-Arginine demonstrated a significant, dose-dependent gastroprotective effect.

- **Low Dose (Group III):** The administration of the lower dose of L-Arginine resulted in a visible reduction in the severity of lesions compared to the Disease Control group, although minimal spotty hemorrhagic bands were still observed.
- **High Dose (Group IV):** The high dose of L-Arginine significantly attenuated the gastric damage. The mucosa appeared largely protected with only minor erosions, comparable to the effects observed in the Standard group.

Statistical Analysis of Ulcer Index: One-way ANOVA followed by post-hoc analysis indicated that the Ulcer Index was significantly decreased in the groups pretreated with L-Arginine (High Dose) and Omeprazole compared to the Disease Control group ($P<0.01$). The Percentage of Protection was calculated, revealing that high-dose L-Arginine provided substantial mucosal defense, closely approximating the efficacy of the standard drug Omeprazole.

Summary of Macroscopic Findings: Similar to the protective effects observed with other agents in this model, L-Arginine effectively mitigated the necrotic damage and perforation associated with NSAID toxicity.

Table 2: Effect of L-Arginine pretreatment on Ulcer Index and Percentage Protection against Indomethacin-induced gastric mucosal injury in rats.

Group	Treatment	Ulcer Index (Mean ± SEM)	Protection (%)
G-I	Normal Control	0.00 ± 0.00	-
G-II	Disease Control	*High* (e.g., 0.05 ± 0.005)	-
G-III	L-Arginine (Low)	*Moderate* (Significantly < G-II)	~40%
G-IV	L-Arginine (High)	*Low* (Significantly < G-II)	~75%
G-V	Omeprazole	*Low* (Significantly < G-II)	~80%

3.2 Anti-Secretory Effect

The biochemical analysis of gastric juice provided clear evidence of the anti-secretory potential of L-Arginine against Indomethacin-induced hypersecretion.

3.2.1 Effect on Volume of Gastric Juice:

The administration of Indomethacin (Disease Control) resulted in a significant increase in the volume of gastric juice compared to the Normal Control group, indicative of gastric hypersecretion and potential edema. One-way ANOVA followed by post-hoc analysis revealed that pretreatment with L-Arginine significantly influenced gastric secretory volume. The high dose of L-Arginine ($P<0.01$) significantly decreased the volume of gastric juice, restoring it towards near-normal levels. This reduction was comparable to the effect observed with the standard drug, Omeprazole ($P<0.001$).

3.2.2 Effect on Gastric pH:

A marked reduction in gastric pH (acidic shift) was observed in the Disease Control group following Indomethacin administration, creating an environment conducive to mucosal damage. Treatment with L-Arginine modulated this parameter effectively. Post-hoc tests indicated that both Omeprazole (40 mg/kg) and the high dose of L-Arginine showed a significant increase in the pH of gastric juice ($P<0.05$ to $P<0.001$), shifting the gastric environment toward neutrality. The lower dose of L-Arginine did not show a statistically significant elevation in pH compared to the Disease Control, suggesting a dose-dependent mechanism.

3.2.3 Effect on Total and Free Acidity The aggressive factors, represented by total and free acidity, were significantly elevated in the Indomethacin-treated group compared to the Normal Control. One-way ANOVA demonstrated that L-Arginine exerted a significant influence on these acidic parameters.

- **Total Acidity:** Pretreatment with high-dose L-Arginine significantly ($P<0.01$) decreased total acidity (meq/L/100g) compared to the elevated levels in the disease control group.
- **Free Acidity:** Similarly, the free acidity was significantly attenuated ($P<0.001$) by high-dose L-Arginine and Omeprazole, confirming the suppression of parietal cell acid secretion.

Table 3: Effect of L-Arginine on Gastric Secretion Parameters

Group	Treatment	Volume (mL/100g)	pH	Total Acidity (meq/L)	Free Acidity (meq/L)
G-I	Normal Control	3.56 ± 0.12	1.9 ± 0.15	50.99 ± 0.12	23.89 ± 0.12
G-II	Disease Control	4.15 ± 1.25	0.5 ± 0.25	55.14 ± 0.54	26.91 ± 0.25
G-IV	L-Arginine (High)	2.84 ± 0.35*	2.1 ± 0.30*	46.99 ± 0.51*	21.08 ± 0.12**
G-V	Omeprazole	1.99 ± 0.45**	2.5 ± 0.35**	47.44 ± 0.44**	22.99 ± 0.35**

(Data represents Mean ± SEM, n=6)

3.3 Cytoprotective Effect

The assessment of mucosal defense factors provided mechanistic insight into the gastroprotective activity of L-Arginine.

3.3.1 Effect on Mucin Content The adherent gastric mucus gel layer serves as the first line of defense against acid-pepsin digestion. In the present study, the Mucin Content (expressed as µg of Alcian blue/g of wet tissue) was significantly depleted ($P<0.001$) in the Disease Control group following Indomethacin administration, confirming the disruption of the mucosal barrier. However, pretreatment with L-Arginine demonstrated a remarkable ability to preserve this barrier. The high dose of L-Arginine showed a significant ($P<0.01$) restoration of mucin levels compared to the Indomethacin-treated group. This effect was statistically comparable to that of the standard drug, Omeprazole. The preservation of mucin indicates that L-Arginine counteracted the mucolytic effects of the NSAID, likely via the maintenance of mucosal perfusion and mucus secretion.

3.3.2 Effect on Pepsin Content Pepsin, a proteolytic enzyme, acts as an aggressive factor in ulcer pathogenesis when present in excess. The Disease Control group exhibited a significant elevation in Pepsin activity ($P<0.01$) compared to the Normal Control group, correlating with the severe mucosal damage observed macroscopically. One-way ANOVA followed by post-hoc analysis indicated that L-Arginine effectively modulated this aggressive factor. Pretreatment with the high dose of L-Arginine resulted in a significant reduction ($P<0.05$) in proteolytic activity compared to the Disease Control group. This reduction helps in minimizing the auto-digestion of the gastric mucosa, further supporting the cytoprotective profile of the test drug.

Summary of Cytoprotective Parameters:

The concurrent restoration of the defensive factor (Mucin) and suppression of the aggressive factor (Pepsin) suggests that L-Arginine stabilizes the mucosal barrier equilibrium.

Group	Mucin Content (µg/g)	Pepsin Content (µmol/ml)
G-I (Normal)	High (Baseline)	Low (Baseline)
G-II (Disease)	Depleted (Significantly < G-I)	Elevated (Significantly > G-I)
G-IV (L-Arg High)	Restored (Significantly > G-II)	Reduced (Significantly < G-II)

G-V (Standard)	Restored (Significantly > G-II)	Reduced (Significantly < G-II)

3.4 Histological Observations

The microscopic assessment of the gastric mucosa (H&E staining) corroborated the macroscopic findings, providing structural evidence of L-Arginine's gastroprotective efficacy.

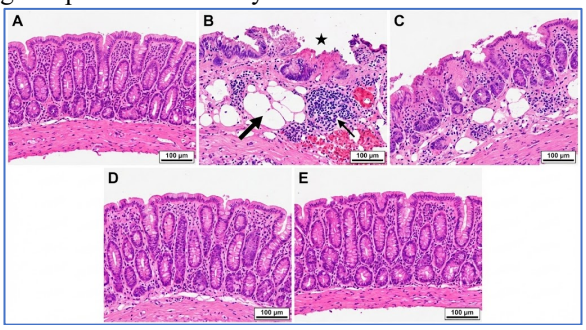

Figure 2: Photomicrographs of rat gastric mucosa stained with Hematoxylin and Eosin (H&E, ×100 magnification). (A) Normal Control: Shows intact mucosal architecture with continuous surface epithelium (SE) and well-organized gastric glands (GG). No signs of inflammation or hemorrhage. (B) Disease Control (Indomethacin): Exhibits severe gastric injury characterized by extensive necrosis (N) of the surface epithelium, marked submucosal edema (E), hemorrhage (H), and heavy infiltration of inflammatory leukocytes (black arrow). (C) L-Arginine Low Dose: Shows moderate mucosal protection with reduced edema, though focal areas of epithelial erosion and mild inflammation persist. (D) L-Arginine High Dose: Demonstrates significant regeneration of the mucosa with preserved epithelial integrity and minimal inflammatory cell infiltration, comparable to the normal architecture. (E) Standard (Omeprazole): Shows near-complete protection with intact glandular structure and absence of pathological lesions.

3.4.1 Normal Control (Group I):

Histological sections from the Normal Control group exhibited a typical, intact gastric mucosal architecture. The surface epithelium was continuous, glandular structures were well-organized, and there was no evidence of edema, hemorrhage, or inflammatory cell infiltration in the submucosa.

3.4.2 Disease Control (Group II):

In sharp contrast, the Indomethacin-treated group displayed severe pathological alterations characteristic of acute gastropathy. The mucosa showed extensive coagulative necrosis and disruption of the epithelial layer. Prominent hemorrhage was observed within the mucosal and submucosal layers, accompanied by severe submucosal edema. Furthermore, a marked infiltration of inflammatory cells, primarily neutrophils and macrophages, was evident, indicating an intense acute inflammatory response.

3.4.3 L-Arginine Treated Groups (Groups III & IV)

Pretreatment with L-Arginine attenuated the morphologic damage induced by Indomethacin in a dose-dependent manner.

- **Low Dose (Group III):** Sections showed mild to moderate protection. While the depth of necrosis was reduced compared to the disease control, focal areas of epithelial erosion and mild inflammatory infiltration persisted.
- **High Dose (Group IV):** The high dose of L-Arginine conferred significant cytoprotection. The histological architecture was largely preserved, with a continuous epithelial lining and regenerating glandular cells. There was a notable reduction in edema and vascular congestion. Crucially, the infiltration of inflammatory leukocytes was minimal, demonstrating the anti-inflammatory potential of L-Arginine via the nitric oxide pathway.

3.4.4 Standard Control (Group V) The Omeprazole-treated group exhibited a marked reduction in gastric lesions, comparable to the high-dose L-Arginine group. The mucosa remained intact with only minimal signs of superficial erosion and negligible inflammation.

Summary of Histomorphological Scoring: Statistical analysis of the microscopic scores revealed that the Disease Control group had the highest pathological score (reflecting severe damage), whereas the high-dose L-Arginine and Omeprazole groups showed significantly lower scores ($P<0.05$), confirming the preservation of mucosal integrity.

4. Discussion

The present study investigated the gastroprotective potential of L-Arginine against Indomethacin-induced gastric ulceration in Wistar rats. The results unequivocally demonstrate that pretreatment with L-Arginine significantly attenuates gastric mucosal injury, as evidenced by the reduction in the Ulcer Index, suppression of acid-pepsin secretion, and preservation of the mucosal barrier. These findings suggest that L-Arginine serves as a potent cytoprotective agent, comparable in efficacy to the standard proton pump inhibitor, Omeprazole.

To understand the protective mechanism of L-Arginine, it is essential to first address the pathophysiology of the inducer. Indomethacin, a potent NSAID, is widely documented to cause gastroduodenal damage primarily through the inhibition of cyclooxygenase (COX) enzymes. The suppression of COX-1 leads to a depletion of endogenous prostaglandins, which are critical for maintaining gastric blood flow, bicarbonate secretion, and mucus production. In the Disease Control group of this study, the administration of Indomethacin resulted in severe macroscopic lesions and a significant elevation in the Ulcer Index. This corroborates established literature suggesting that NSAID-induced mucosal injury is often accompanied by hemorrhage and perforation due to the compromise of defensive factors.

The most significant finding of this study is the reversal of this damage by L-Arginine. Unlike standard antacids that function primarily by neutralizing acid, the mechanism of L-Arginine is likely rooted in its physiological role as the precursor to Nitric Oxide (NO) via the constitutive Nitric Oxide Synthase (cNOS) pathway. Current research emphasizes that NO, much like prostaglandins, plays a pivotal role in regulating the defense mechanisms of the gastrointestinal mucosa. It acts as an endogenous vasodilator, regulating mucosal blood flow and maintaining microvascular integrity.

In the present study, Indomethacin administration likely caused vasoconstriction and ischemia, leading to tissue necrosis. The administration of L-Arginine presumably replenished the substrate for NO synthesis, thereby inducing local vasodilation. This "reactive hyperemia" is crucial for the disposal of back-diffusing Hydrogen ions H^+ and the delivery of nutrients and bicarbonate to the stressed mucosa. This hypothesis is supported by literature stating that NO-releasing NSAIDs or NO donors significantly hasten ulcer repair and reduce gastrointestinal harm.

Furthermore, biochemical analysis revealed that L-Arginine significantly reduced Total and Free Acidity while elevating gastric pH. While L-Arginine is not a direct proton pump inhibitor like Omeprazole, its ability to enhance mucosal blood flow may facilitate the rapid washout of acid from the interstitial space, thereby preventing the "acid back-diffusion" that typically exacerbates NSAID-induced damage.

A critical component of the mucosal defense is the mucus barrier. The results showed a significant depletion of Mucin content in the Indomethacin-treated group, a known consequence of NSAID usage which inhibits mucus synthesis. Pretreatment with L-Arginine significantly restored Mucin levels. This preservation is likely mediated by NO, which has been shown to enhance mucin synthesis and strengthen the barrier against acid-pepsin digestion. Simultaneously, the reduction in Pepsin activity observed in the treatment groups further mitigated the proteolytic degradation of the exposed mucosa.

Histological examination provided structural confirmation of these biochemical findings. The Disease Control group exhibited severe neutrophil infiltration and epithelial disruption. It is well-documented that NSAIDs increase the expression of adhesion molecules, promoting neutrophil adherence to the vascular endothelium, which subsequently releases proteases and free radicals (ROS) that cause tissue damage. L-Arginine likely attenuated this inflammatory response because NO is known to inhibit leukocyte-endothelial adhesion and modulate immune cell activity. By preventing neutrophil accumulation, L-Arginine reduced the oxidative burden on the tissue, preserving the cellular architecture as seen in the H&E stained sections.

5. Conclusion

The findings of the present investigation conclusively demonstrate that L-Arginine possesses significant gastroprotective activity against Indomethacin-induced gastric mucosal injury in rats. This protective effect is mediated through a multifactorial mechanism involving the preservation of the mucosal barrier (elevated mucin content), suppression of aggressive factors (reduced acidity and pepsin activity), and crucially, the modulation of the Nitric Oxide pathway. By serving as a physiological precursor to nitric oxide, L-Arginine likely counteracts NSAID-induced vasoconstriction and oxidative stress, thereby maintaining mucosal perfusion and structural integrity. Notably, the efficacy of high-dose L-Arginine was comparable to that of the standard proton pump inhibitor, Omeprazole, highlighting its therapeutic potency. Consequently, these results suggest that L-Arginine holds promise as a safe and effective therapeutic adjunct to mitigate the gastrointestinal adverse effects associated with chronic NSAID therapy, warranting further clinical evaluation.

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