Type of the Paper (Research article)

A Comparative Study on the Effects of Lactobacillus Acidophilus, Chlorella, and Esomeprazole on Indomethacin-Induced Gastric Ulcer in Adult Albino Rats

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Received: 10 March, 2025 Reviewed: 6 April, 2025
Accepted: 23 June, 2025 Published online: 20 September 2025

Abstract:

Introduction: Gastric ulcers are among the most prevalent gastrointestinal disorders. Both *Lactobacillus acidophilus* and Chlorella contain natural antioxidants and anti-inflammatory agents.

Aim of the study: To investigate the possibility of protective properties of *Chlorella* and *Lactobacillus acidophilus* against indomethacin-induced stomach ulcers in adult albino rats compared to the usual treatment (Esomeprazole).

Methods: Five groups were created from 25 mature male albino rats: Groups I (control) and II (only indomethacin): Oral 100 mg/kg, Group III (Esomeprazole + Indomethacin): 5 mg/kg Esomeprazole orally, Group IV (Chlorella + Indomethacin): 2000 mg/kg Chlorella orally, Group V (*Lactobacillus acidophilus* + Indomethacin): 10 billion CFU/rat orally. For ten days in a row, all rats in the experimental groups got their preventative medications, then on day 11, they were all given a single oral dosage of indomethacin. Six hours after indomethacin administration, the animals were euthanized, and their stomachs were harvested for ulcer index calculations, immunohistochemical labeling (NF-κB), and histological analysis (PAS reaction, H&E staining). A morphometric analysis (area percentage of PAS and NF-κB) was performed, followed by statistical evaluation.

Results: When compared to control, the intake of indomethacin led to a significant increase in NF-κB expression, a significant decrease in PAS reactivity, and greater ulcer indicators. These disturbances were considerably lessened by pre-treatment with Esomeprazole, Chlorella, or *Lactobacillus acidophilus.* Interestingly, the most noticeable morphometric and histological improvements were seen by the *Lactobacillus acidophilus* group.

Conclusions: Both Chlorella and *Lactobacillus acidophilus* offered substantial protective effects against indomethacin-induced gastric mucosal damage, exceeding those seen with Esomeprazole alone. Lactobacillus acidophilus exhibited a stronger prophylactic impact than Chlorella.

Keywords: Indomethacin; Gastric Ulcer; Chlorella; *Lactobacillus acidophilus*; Esomeprazole; NF-κB.

1. Introduction

Nearly 5% of people worldwide suffer from gastric ulcers, a common gastrointestinal ailment. An imbalance between the stomach's natural defenses and aggressive elements like gastric acid and pepsin causes these ulcers by damaging the mucosa and causing inflammation. Non-steroidal anti-inflammatory medicines (NSAIDs) are well known for their ulcerogenic effects and are one of the major causes of stomach mucosal damage [1].

Indomethacin, an indole-derived substance with high gastrointestinal absorption after oral dosing, is one of the most often utilized NSAIDs in experimental ulcer models.

Once absorbed, Indomethacin undergoes metabolic conversion, forming active metabolites that significantly contribute to ulcer development by impairing gastric mucosal defense mechanisms [2,3]. To counteract the effects of excessive gastric acid secretion, proton pump inhibitors (PPIs) such as Esomeprazole (ESP) are commonly prescribed. These medications are known for their potent acid-suppressive effects, which aid in reducing gastric acidity and accelerating ulcer healing [4]. Despite their efficacy in ulcer treatment, long-term PPI usage has been associated with potential adverse effects. Several studies have reported a possible link between prolonged esomeprazole consumption and an increased risk of developing dementia and kidney dysfunction [5]. Due to these concerns, researchers have been exploring alternative therapies, particularly plant-derived bioactive compounds, which have demonstrated promising potential in disease prevention and treatment. According to the World Health Organization (WHO), 10% of individuals worldwide manage digestive problems with herbal medications [6].

Chlorella, a unicellular green alga that is rich in proteins, fibers, essential fatty acids, vitamins, minerals, and phytonutrients, is one promising natural compound with gastroprotective potential. In addition to its nutritional advantages, Chlorella has drawn interest for its pharmacological uses, especially in the treatment of peptic ulcers [7].

Various studies have demonstrated that Chlorella’s bioactive molecules possess antimicrobial properties, anticancer potential in breast cancer models, glucose and lipid regulation in obesity, antihypertensive

activity, and tumor-suppressive effects [8]. Additionally, a previous study reported that Chlorella supplementation contributes to lowering blood pressure and cholesterol levels, accelerating wound healing, and boosting immune function. It has also been recognized for its role in relieving symptoms of fibromyalgia, hypertension, and ulcerative colitis, making it a multifunctional therapeutic agent. The use of probiotics, which the Food and Agriculture Organization (FAO) and WHO define as living microorganisms that offer health advantages when taken in sufficient proportions, is another possible gastrointestinal therapeutic [10].

These beneficial microbes help in modulating gut microbiota, thus supporting immune function and maintaining overall digestive health [11]. Probiotics, when administered in sufficient concentrations, are regarded as non-pathogenic, non-carcinogenic, and non-invasive. Their key function involves temporarily colonizing the gastrointestinal tract, where they suppress the proliferation of harmful bacteria while maintaining microbial balance [12]. Considering the gastroprotective potential of Chlorella and probiotics, this study aims to investigate their effects in preventing Indomethacin-induced gastric ulcers and compare their protective benefits with those of Esomeprazole, a widely used antiulcer treatment.

2. Subjects & Methods

2.1. Subjects

Twenty-five mature male albino rats, weighing between 220 and 280 grams and about four months of age, participated in this investigation. Within the Faculty of Science at Fayoum University, the animals were kept in clean, well-ventilated stainless-steel cages. They were provided with ad libitum, regular laboratory feed, and tap water.

2.2.Methods

Drugs

- Indomethacin: (Indocid capsules) supplied from Kahira Pharmaceutical & Chemical Industries Company. Each capsule contained 25 mg indomethacin.
- Esomeprazole: (Esmorap capsules) supplied from AUG Pharma. Each capsule contained 40 mg esomeprazole.

- Chlorella: supplied from the NOW Foods company, U.S.A., in powder form.
- Probiotics (*Lactobacillus acidophilus*): (Lacteol fort sachets) supplied from Tenth of Ramadan for pharmaceutical industries & diagnostic reagent (rameda) 6th October City, A.R.E. Each sachet contains 10 billion CFU.

Assessment of ESP, Chlorella, and LB's recurrent acute drug toxicity

The acute toxicity of Esomeprazole, Chlorella, and *Lactobacillus acidophilus* was assessed following the Nuffield Council on Bioethics guidelines [13]. To guarantee fasting conditions, food was removed for a full day before to testing. After that, the animals were split up into three groups, each consisting of ten rats. For ten days in a row, each group was given an oral dosage of 2000 mg/kg of Chlorella, 10 billion CFU/rat of *Lactobacillus acidophilus*, or 5 mg/kg of Esomeprazole.

Design of experiments

Five experimental groups, each with five rats, were randomly assigned to the animals:

- Group I (Control): Three rats were given 1 milliliter of distilled water, which serves as a solvent for the test substances, once a day for 11 days, while two rats were assigned to the normal control group.
- Group II (group treated exclusively with Indomethacin): On Day 11, each rat was given a single oral dose of Indomethacin (100 mg/kg, dissolved in 1 ml of distilled water) through an intragastric gavage tube. Six hours following administration, the animals were slaughtered [1].
- Group III (Esomeprazole pre-treated group): Each rat received a daily oral dose of 5 mg/kg (1 ml/rat) via intragastric gavage for 10 days in a row after a 40 mg capsule of Esomeprazole was dissolved in 32 ml of distilled water. On Day 11, the last dose of Esomeprazole was given two hours before the intake of Indomethacin [14].
- Group IV (Chlorella pre-treated group): 500 mg of chlorella powder was dissolved in 2 ml of distilled water, and each rat received a daily oral dose of 2000 mg/kg (2 ml/rat) via intragastric gavage for 10 days in a row. On Day 11, the last chlorella dose was given two hours before the intake of Indomethacin [15].
- Group V (*Lactobacillus acidophilus* pre-treated group): For 10 days, each rat was given an oral dose of 10 billion CFU/rat (1 ml/rat) by intragastric gavage after a 10 billion CFU sachet of *Lactobacillus*

acidophilus was dissolved in 1 ml of distilled water. On Day 11, the last dose of *Lactobacillus acidophilus* was given two hours before the administration of Indomethacin [16].

Rats were given free access to water but fasted for 24 hours before ulcer induction. Exactly two hours after the previous preventive therapy, indomethacin (100 mg/kg) was administered orally as a single dosage to prevent gastric ulcers. Six hours after the indomethacin was administered, the presence of stomach ulcers was evaluated [1].

Sample Collection for Histopathological Analysis

In order to anesthetize all of the rats, thiopental sodium (50 mg/kg) was given intraperitoneally on Day 11 of the experiment, six hours following indomethacin treatment.

Following the extraction of the stomach tissues, they were saline-rinsed and dissected along the greater curvature. Using a digital camera, the mucosal surface was positioned upward and captured on camera. A calculation of the ulcer index was made. Immediately after being fixed in 10% buffered formalin, stomach specimens were prepared for paraffin embedding. Thin serial sections, 5 μm thick, were made in preparation for immunohistochemical and histological staining methods.

Measurement of ulcer index

A magnifying eyepiece on a microscope was used to evaluate the ulcer. The ulcer score was determined by averaging the number of stomach ulcers in each group. Each rat's ulcer score was multiplied by 100 to determine the ulcer index (UI). The ulcer score was calculated using the average number of stomach ulcers in the rats in each group (**Table 1**). The ulcer score of each rat was then multiplied by 100 to determine the ulcer index (UI). Subsequently, the ulcer group's UI minus the treated group's UI was divided by 100% to get the inhibition index, or percentage of ulcer inhibition [6].

Table 1: Descriptive features of Ulcer scores.

Score	Features
0	No ulcer
1	Identify pinpoint ulcers and alterations that are restricted to the mucosa's outer layers.
2	A less than 1 mm in size ulcer
3	An ulcer that is larger than 1 mm but smaller than 2 mm
4	Perforated ulcer or ulcer larger than 2 mm

Histopathology

Following a careful dissection of the stomach, each rat's fundic area was immediately preserved in 10% formalin for 24 hours to preserve tissue integrity before further processing for histopathological evaluation. Following the collection of tissue samples, thin sections (5 µm thick) were produced for microscopic inspection and fixed in paraffin wax. Hematoxylin and eosin (H&E) staining was applied to these slices to evaluate general histological features. and examined for mucus content using Periodic Acid–Schiff (PAS) staining. Additionally, NF-κB expression was investigated by immunohistochemistry labeling using a rat polyclonal antibody (Bioss Antibodies, USA; catalog no. BS-20159R).

Morphometric study

Quantitative image analysis was conducted using the "Top View" image analyzer system (China) at the Histology Department, Faculty of Medicine, Fayoum University. The system comprised a color video camera (Olympus), A color monitor, an IBM personal computer, and a light microscope (Olympus CXL-47, Olympus Optical Co. Ltd., Japan) equipped with a digital video camera (Olympus C5050). The image analyzer was automatically calibrated to convert pixel measurements into micrometers (µm) units.

The following morphometric parameters were evaluated

Mucosal Fold Depth: Measured in ten non-overlapping fields per section using a 20× objective lens (total magnification 200×). PAS Reaction Area Percentage: Evaluated in ten non-overlapping fields

using a 20× objective lens (total magnification 200×). NF-κB Immunoreactivity Area Percentage: Assessed in ten non-overlapping fields per section using a 40× objective lens (total magnification 400×).

2.3. Analysis of Statistics

To evaluate group differences, the morphometric data were gathered, arranged in tables, and examined. Before statistical analysis, the normality of the data distribution was confirmed. To compare the experimental groups, the analysis used analysis of variance (ANOVA) and standard deviation (SD). *P*-values less than 0.05 were regarded as statistically significant. SPSS for Windows, Version 27, was used to do statistical calculations [17].

3. Results

3.1. Ulcer index and inhibition index

In the Indomethacin group, rats exhibited a high ulcer score (27.4 ± 7.2) and an ulcer index (UI) of 2740, indicating severe gastric damage. Pre-treatment with Esomeprazole, Chlorella, or *Lactobacillus acidophilus* before indomethacin intake significantly reduced ulcer severity. Among the tested protective agents, *Lactobacillus acidophilus* demonstrated the strongest gastroprotective effect, with an ulcer index of 820 and a net preventive index of 70%. Chlorella-pretreated rats exhibited a UI of 1060, yielding a 61.3% preventive index. Rats given esomeprazole had the lowest protective effect, with a 51.8% preventative index and a UI of 1320 (**Table 2**).

Table 2: Mean ± SD of UC, UI, and the net preventative index.

Groups	Ulcer score	Ulcer index	Net preventive index
Control	-----	----	----
INDO	27.4 ± 7.2	2740*	0
Esomeprazole + INDO	13.2 ± 3.2	1320*#	51.8%
Chlorella + INDO	10.6 ± 1.5	1060*#	61.3%
LB + INDO	8.2 ± 2.2	820*#	70%

** A significant difference from Control is shown by P < 0.05, while # a significant difference from INDO is indicated by P < 0.05.*

3.2. Histological results

Gross examination

Macroscopic images of the gastric mucosa revealed ulcerations of varying severity in the Indomethacin-treated group and all pretreated groups, while the control group exhibited intact mucosal surfaces **(Figure 1).**

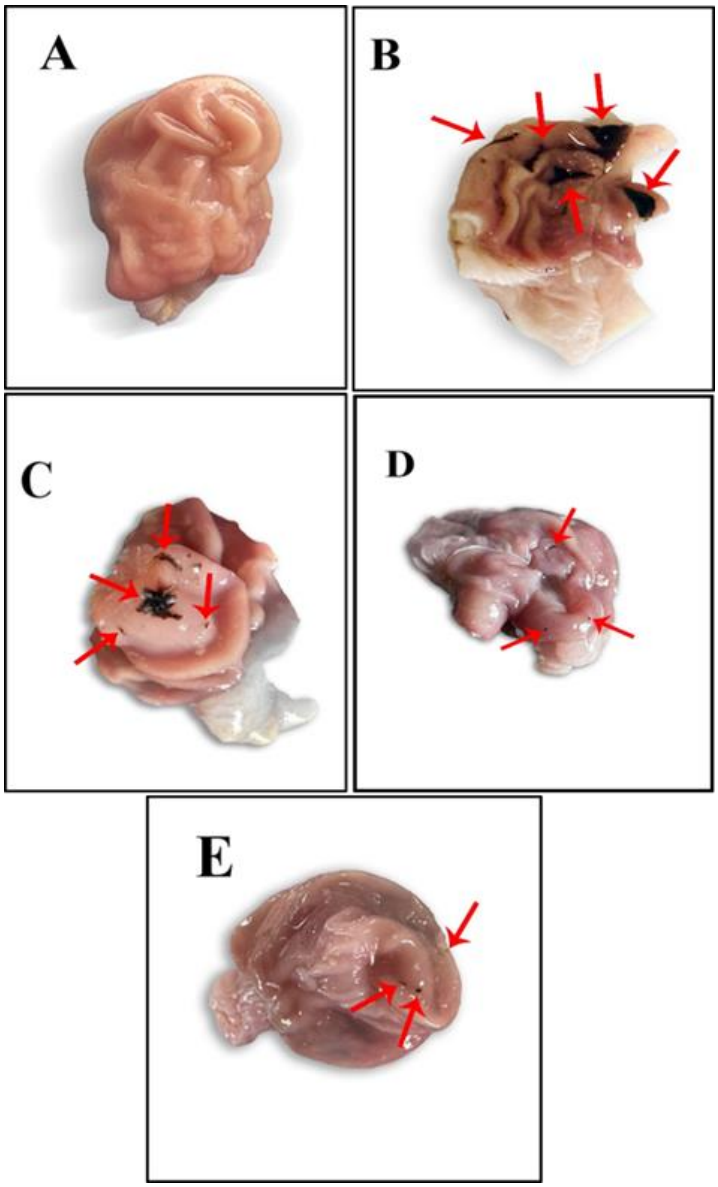

Figure 1: Gross images of each group's stomach mucosa under examination: A) control group, with intact mucosa, B) Indo-only treated group showing high severity of ulcerations (arrows), while Esomeprazole pre-treated group, C) Chlorella pre-treated group, D) LB pre-treated group, E) showed less severity (arrows).

3.3. Hematoxylin & Eosin (H&E) Results

Histological analysis of the fundic region in the control group revealed intact mucosal architecture, with fundic glands which is tubular, arranged perpendicular to the epithelium surface. Gastric pits were observed as short and narrow structures, with glands extending deep into the lamina propria **(Figure 2a)**. Surface mucus-secreting cells were columnar in shape, possessing oval nuclei located at the basal region. Parietal cells had a polygonal appearance, with vesicular nuclei and acidophilic cytoplasm **(Figure 2b)**. Chief (peptic) cells, located at the glandular base, exhibited basophilic cytoplasm **(Figure 2c).**

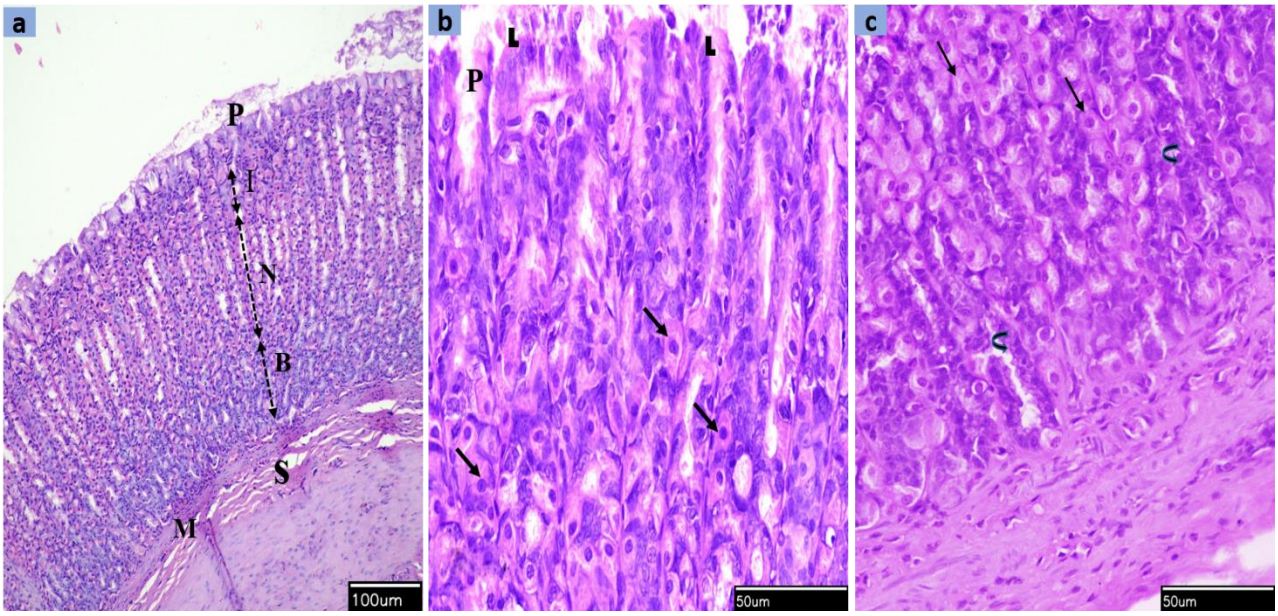

Figure 2: Photomicrographs of fundic sections from control rats' gastric mucosa, illustrating the normal histological structure: **(a)** The tubular fundic glands are uniformly arranged, oriented at a right angle to the epithelium surface, and feature narrow, short pits (P). There are three separate zones within the glands: the outer base (B) middle neck (N), and the inner isthmus (I), all of which are embedded within the lamina propria down to the muscularis mucosa (M) and submucosa (S), which are also visible. **(b)** Surface mucus-secreting cells appear columnar, with basally positioned oval nuclei (arrowhead). Parietal cells exhibit acidophilic cytoplasm, vesicular nuclei, with a polyhedral shape (arrows). (P) The gastric pits can be easily

identified. (c) The base of the fundic glands displays parietal cells (arrows) and peptic cells with basophilic cytoplasm (curved arrows) in their typical form **(a: H&E, X100, b & c: X400).**

Gastric mucosal sections from INDO-treated rats exhibited disruption of structural organization, with epithelial exfoliation and loss of surface mucus-secreting cells. Certain regions showed mucosal erosion, hemorrhage, and hemosiderin deposition **(Figure 3a).** Parietal cells displayed ballooning and vacuolation, with pyknotic nuclei displaced eccentrically. Observations also included red blood cell extravasation and disorganized glandular bases with densely stained nuclei **(Figures 3b, 3c).** Partial restoration of gastric mucosal integrity was observed in rats pretreated with esomeprazole.

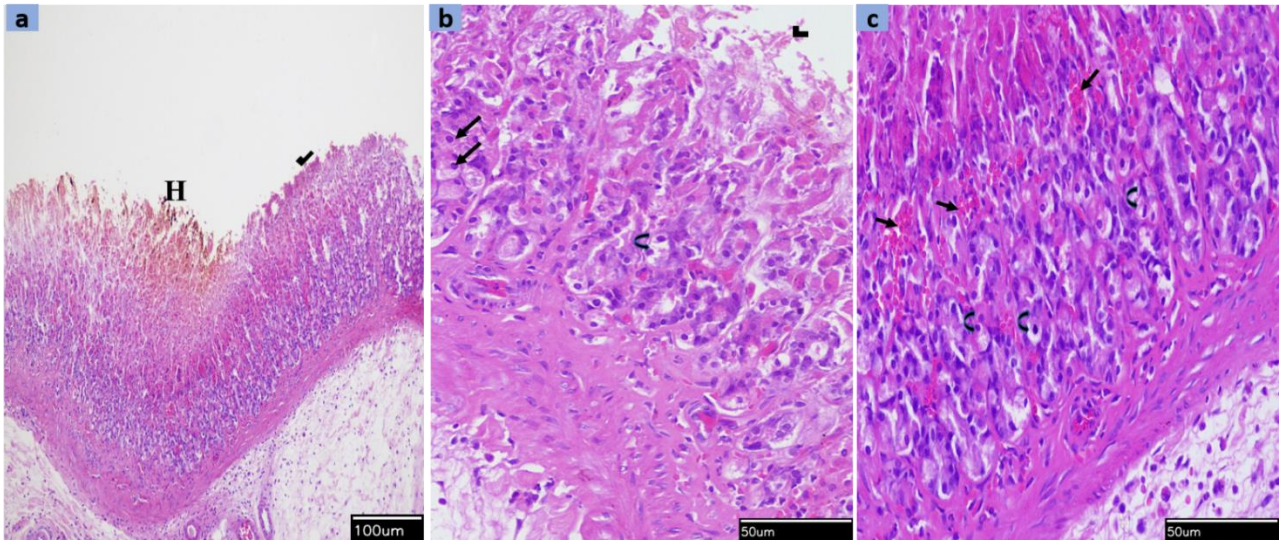

Figure 3: Photomicrographs of fundic sections from the gastric mucosa of Indomethacin (INDO)–treated rats, demonstrating structural alterations and mucosal damage: **(a)** Disruption of the fundic mucosal architecture, characterized by sloughing of the surface epithelium (arrowhead). Evidence of hemorrhage and hemosiderin deposition (H) is observed. **(b)** The fundic mucosa exhibits severe disorganization, with desquamation and further sloughing of the epithelial lining (arrowhead). Parietal cells display ballooning, with eccentric, darkly stained nuclei (arrows), while other cells exhibit vacuolated cytoplasm (curved arrows). **(c)** Glandular bases appear highly disorganized, with dark-stained nuclei. Parietal cells display small, dark nuclei and vacuolated cytoplasm (curved arrow). Extravasation of red blood cells (RBCs) is evident (arrow). **(a: H&E, X100, b & c: X400)**

Fundic glands, which are tubular and extend into the lamina propria, down to the muscularis mucosa, though small areas of mucosal erosion remained. Surface mucus-secreting cells appeared preserved and intact. Several parietal cells exhibited small, dark-stained nuclei with vacuolated cytoplasm. The glandular base retained a relatively normal structure, though some parietal cells still showed dark-stained nuclei and vacuolation **(Figure 4).**

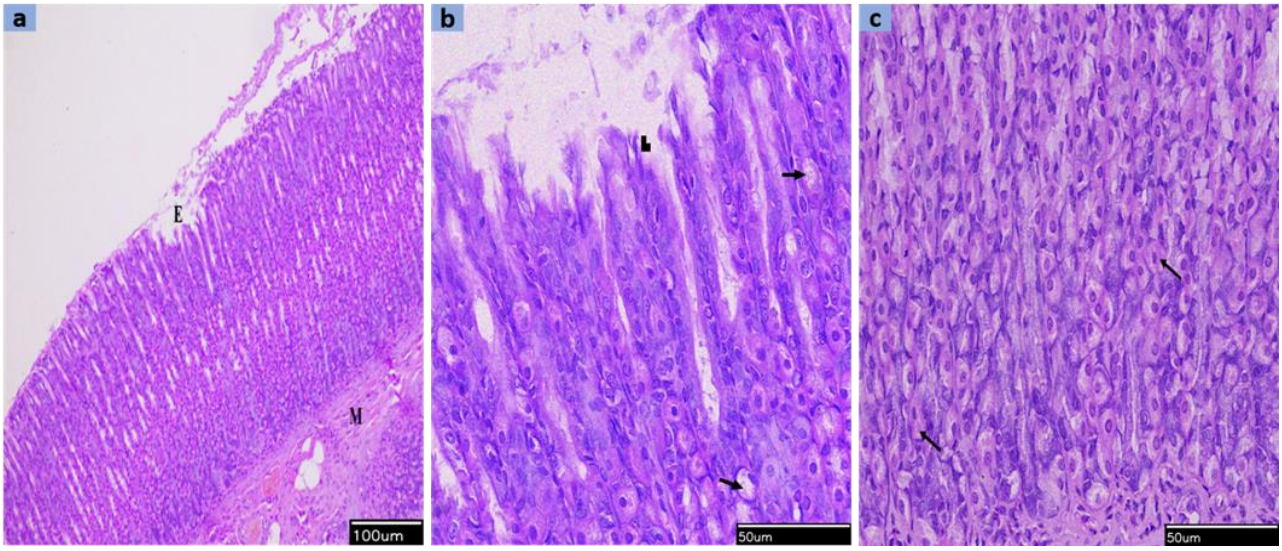

Figure 4: Photomicrographs of fundic sections from esomeprazole (ESP) pre-treated rats' gastric mucosa, illustrating partial restoration of mucosal structure: **(a)** The tubular fundic glands exhibit a restored, organized arrangement, extending into the lamina propria, down to the muscularis mucosa (M). A small area of erosion (E) is still observed. **(b)** Columnar mucus-secreting cells on the surface seem intact and well-organized (arrowhead). Numerous parietal cells have tiny, dark-stained nuclei and cytoplasmic vacuolation (arrows). **(c)** Bases of fundic glands showing partial structural restoration, though some parietal cells still display darkly stained nuclei and vacuolated cytoplasm (arrows). **(a: H&E, X100, b & c: X400)**

Fundic sections from rats pretreated with Chlorella showed a tubular fundic gland configuration that was well-preserved, reaching the muscularis mucosa through the lamina propria. Several parietal cells displayed small, eccentrically positioned dark-stained nuclei with vacuolated cytoplasm, while others appeared morphologically normal. The glandular base showed restoration, with chief and parietal cells exhibiting a typical structure. The lamina propria showed a blood vessel congestion **(Figure 5).**

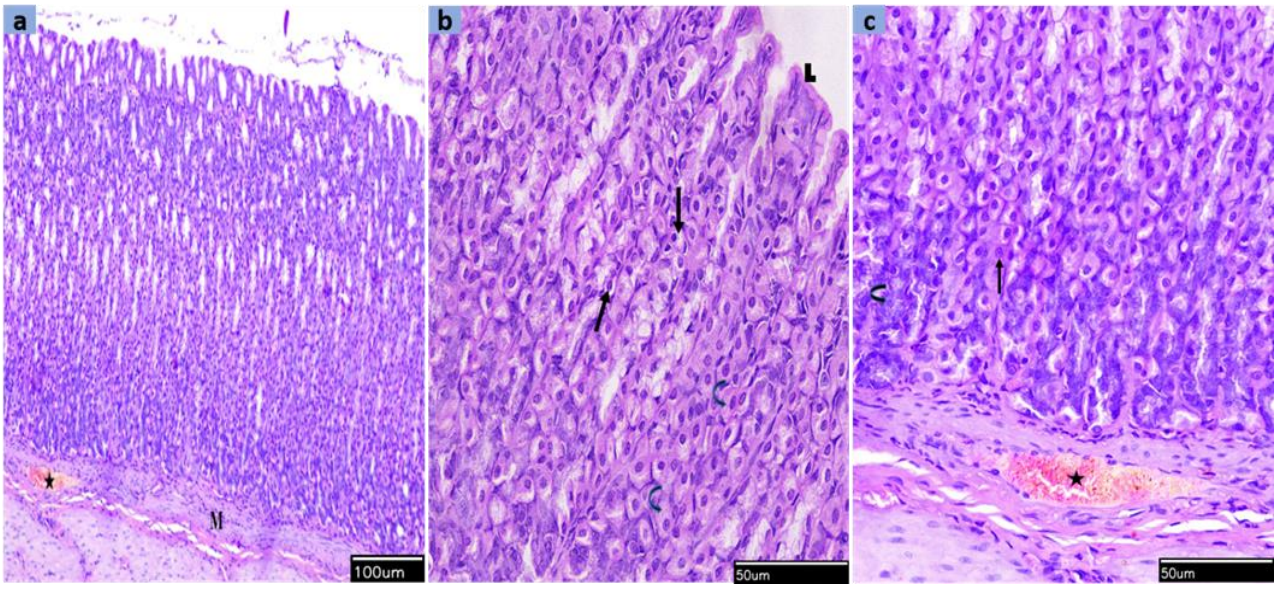

Figure 5: Photomicrographs of fundic sections from the gastric mucosa of Chlorella pre-treated rats, illustrating preserved mucosal structure with mild alterations: **(a)** The tubular fundic glands display a regular, well-organized arrangement, extending into lamina propria, down to the muscularis mucosa (M). Mild glandular widening is observed, along with blood vessel congestion within the lamina propria (star). **(b)** Several parietal cells exhibit small, eccentric, darkly stained nuclei and vacuolated cytoplasm (arrows), while others retain central vesicular nuclei with acidophilic cytoplasm (curved arrows). The surface columnar mucus-secreting cells appear intact and well-structured (arrowhead). **(c)** The fundic gland bases appeared to be restored, with peptic cells maintaining their normal morphology (curved arrow) and parietal cells appearing intact (arrow). Blood vessel congestion is still seen in the lamina propria (star). **(a: H&E, X100, b & c: X400)**

Fundic mucosal sections from rats receiving *Lactobacillus acidophilu*s displayed near-normal histological features, with regularly arranged tubular glands extending into the lamina propria, down to the muscularis mucosa. parietal cells and surface columnar mucus-secreting cells retained a normal structure and distribution. The glandular base appeared restored, with chief and parietal cells displaying typical histological characteristics. Some parietal cells had nuclei that were darkly stained with vacuolated cytoplasm **(Figure 6).**

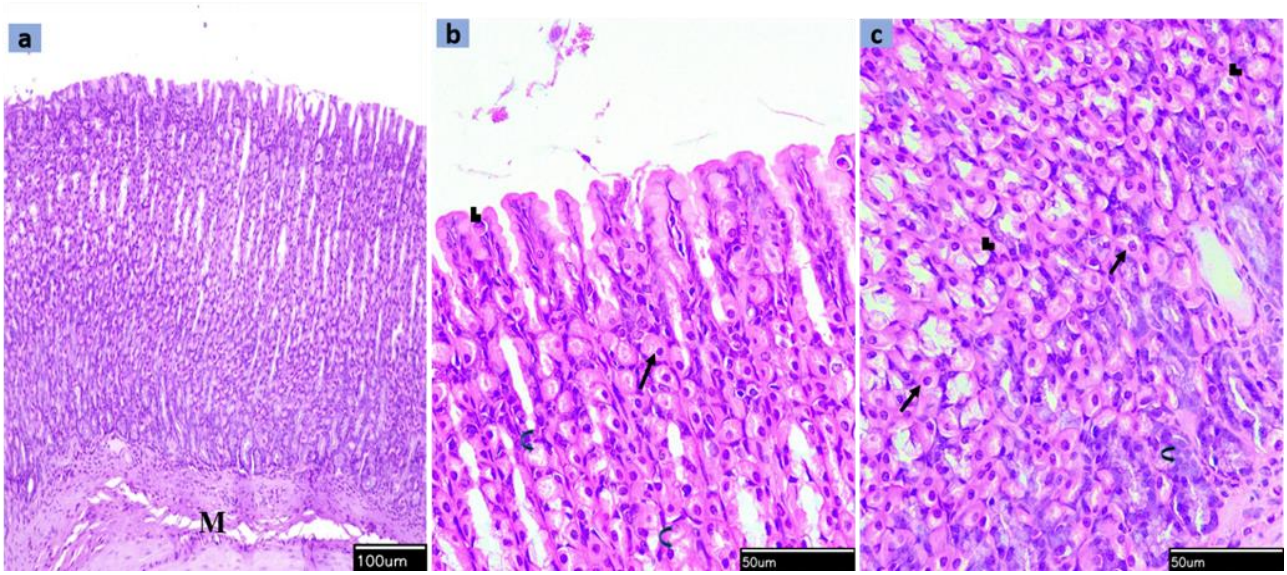

Figure 6: Photomicrographs of fundic sections from Lactobacillus acidophilus (LB) pre-treated rats' gastric mucosa, demonstrating near-normal mucosal structure: **(a)** The tubular fundic glands exhibit a normal and well-organized arrangement, extending into lamina propria, down to the muscularis mucosa (M). **(b):** While parietal cells appear intact (arrows), some of them show vacuolated cytoplasm, small and dark-stained nuclei, and (curved arrows). surface columnar mucus-secreting cells display a regular morphology (arrowhead), **(c)** The fundic gland bases appeared to be restored, with parietal cells (arrows) and peptic (chief) cells (curved arrow) maintaining normal appearance. However, some parietal cells exhibit vacuolated cytoplasm (arrowheads). **(a: H&E, X100, b & c: X400).**

3.4. Periodic acid Schiff's Reaction results

Histological examination of fundic sections revealed that the experimental groups' PAS reactions varied in intensity: The Control group displayed a reliable, strong positive reaction within the neck region and gastric pits **(Figure 7a).** In certain areas, the group treated with indomethacin (INDO) exhibited a negative reaction, while other regions exhibited a very mild positive reaction **(Figure 7b)**. The group which received esomeprazole (ESP) beforehand showed a consistent, significant positive reaction in the stomach pits, but only a weak reaction in the neck area. **(Figure 7c)**. Chlorella & *Lactobacillus acidophilus* (LB) pre-treated groups both displayed a reliable, strong positive reaction in the gastric pits **(Figure 7d, e)**.

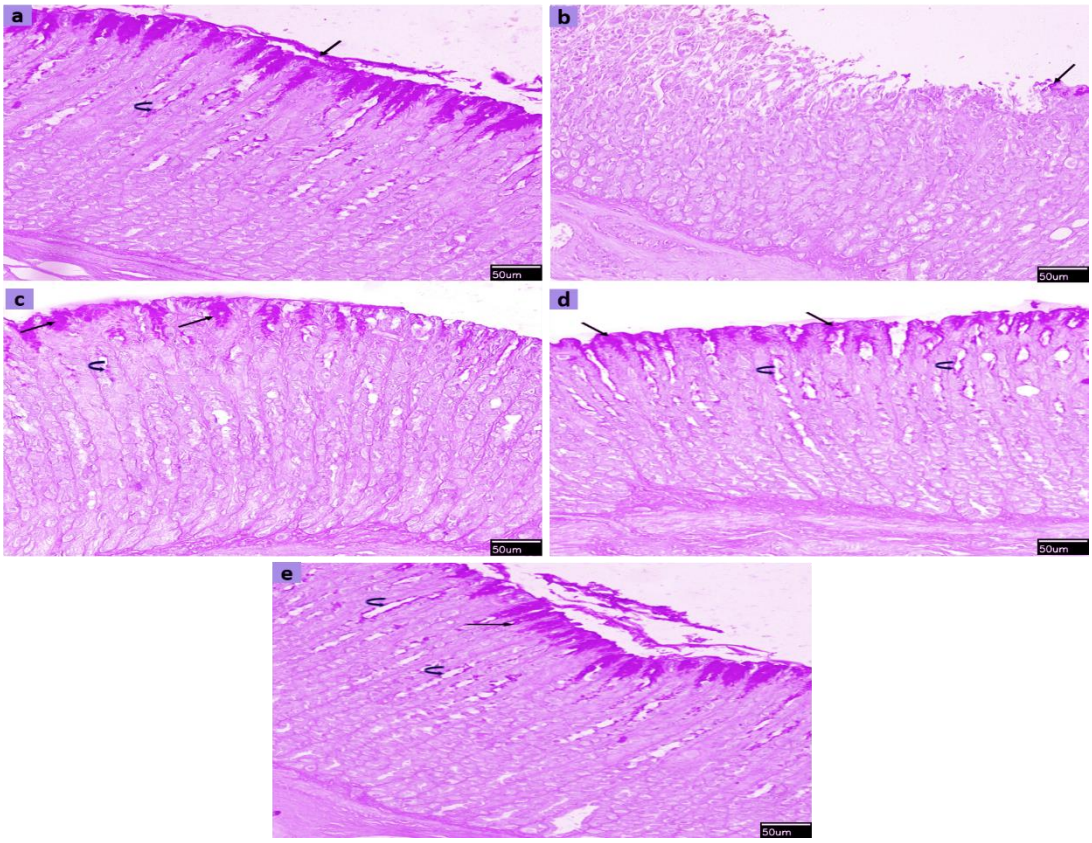

Figure 7: Photomicrographs of fundic sections from the gastric mucosa, demonstrating variations in PAS reaction intensity across different experimental groups: **(a)** Control group displaying a reliable, strong positive reaction in the neck region (curved arrow) and gastric pits (arrow). **(b)** In certain locations, the group treated with indomethacin (INDO) demonstrates a negative reaction, whilst in other areas, there is a very slight positive reaction (arrow). **(c)** stomach pits (arrow) show a consistent, strong positive reaction in the (ESP) pre-treated group, while the neck region shows a mild reaction (curved arrow). **(d)** A dependable, robust positive reaction is shown by the chlorella-pretreated group in the neck area (curved arrows) and the stomach pits (arrow). **(e)** A consistent, robust positive reaction in the neck area (curved arrows) and stomach pits (arrow) is shown by the Lactobacillus acidophilus (LB) pre-treated group **(PAS, X200).**

3.5. NF-κB immunoreaction results

Control rats' fundic sections displayed a negative NF-κB immunohistochemistry reaction **(Figure 8a).** NF-κB cytoplasmic immunohistochemistry reaction was strongly positive in the Indomethacin-treated group **(Figure 8b).** The cytoplasmic immunohistochemistry reaction for NF-κB in the surface epithelium and the basal portion was positive in fundic sections from both the esomeprazole and chlorella pretreatment groups **(Figures 8c, d).** However, a faint positive cytoplasmic immunohistochemistry reaction for NF-κB was observed in the

fundic mucosa of the *Lactobacillus acidophilus* pre-treated group **(Figure 8e).**

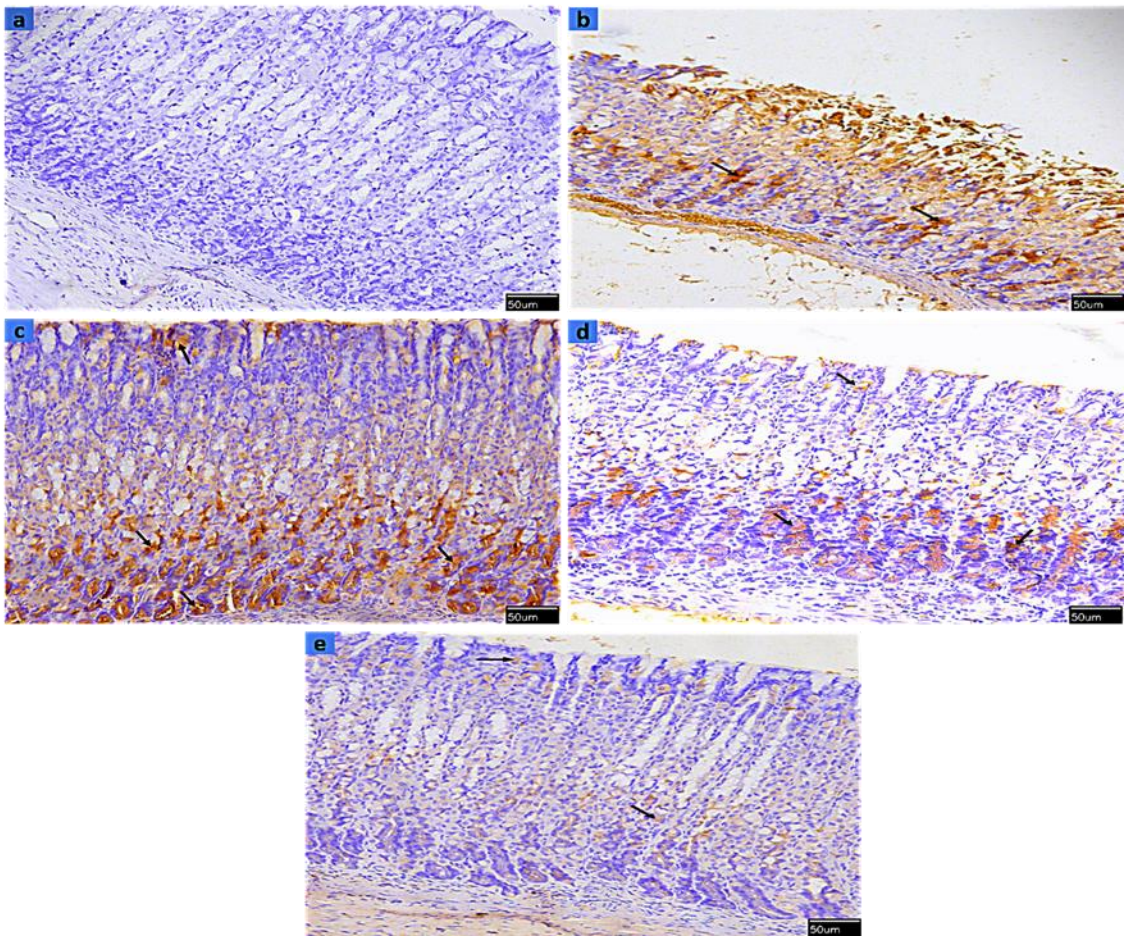

Figure 8: Photomicrographs of fundic sections from **(a)** control rats showing negative immunohistochemical reaction for NF-κB. **(b)** INDO INDO-treated group revealed a strong positive cytoplasmic immunohistochemical reaction for NF-κB (arrows). **(c)** The esomeprazole pre-treated group showed a positive cytoplasmic immunohistochemical reaction for NF-κB in the surface epithelium and the basal part (arrows). **(d)** The Chlorella pre-treated group showed positive cytoplasmic immunohistochemical reaction for NF-κB in the surface epithelium and the basal part (arrows). **(e):** LB pre-treated group revealed a weak positive cytoplasmic immunohistochemical reaction for NF-κB (arrows) **(NF-κB, X200).**

3.6. Morphometric results

Mucosal depth in the INDO group was substantially lower (P <0.05) than in the control and other pre-treated groups. Furthermore, compared to the control group, the Chlorella+INDO group showed a substantial increase in mucosal depth (P < 0.05). In comparison to the control group, there was no discernible change in mucosal

depth in either the ESP+IND or LB+INDO groups (**Table 3**).

Table 3: Mean ± SD of Mucosal depth in micrometre in all examined groups.

Group	Control	Indo	ESP + Indo	Chlorllea + Indo	LB + Indo
Mucosal Depth	323.06 ±22.7	211.96 ±25.04 *	333.42 ±27.01 #	417.44 ±10.09 *#	302.54 ±42.36 #

*ANOVA test results show that the P value is significant at < 0.05. *Noteworthy distinction from Control. # noteworthy distinction from INDO*

Compared to the control group, the INDO group and other pre-treated groups had a significantly decreased area percentage of PAS reaction (P <0.05). Comparing all groups to the INDO group revealed a substantial increase (P < 0.05) in the area % of PAS reaction. Compared to the ESP+INDO and Chlorella +INDO groups, the LB+INDO group exhibited the highest value and a significantly higher area percentage of PAS reaction (P <0.05). The area % of PAS reaction was considerably elevated when the Chlorella +INDO group was contrasted with the ESP+INDO group. The INDO group and other pre-treated groups exhibited a considerably higher area percentage of NF-κB reaction (P <0.05) in comparison to the control group. Comparing the INDO group to all pre-treated groups revealed a substantial decrease in the area percentage of NF-κB reaction (P <0.05). Compared to the ESP+INDO and Chlorella +INDO groups, the LB+INDO group displayed the lowest value and a significantly reduced area percentage of NF-κB reaction (P <0.05). However, this area percentage was much lower in the Chlorella +INDO group than in the ESP+INDO group (**Table 4**).

Table 4: Mean ± SD of area percentage of PAS & NF-κB +ve reaction in all examined groups:

Group	Control	Indo	ESP + Indo	Chlorllea + Indo	LB + Indo
PAS	14.04 ± 0.20	1.77 ± 0.06*	4.30 ± 0.29*#@&	5.72 ± 0.41*#@$	7.38 ± 0.87*#&$
NF-κB	6.33 ± 0.42	23.82 ± 0.20*	16.61 ± 0.38*#@&	13.21 ± 0.23*#@$	10.45 ± 0.46*#&$

*Using the ANOVA test, the P value is significant at less than 0.05. *Considerable variation from Control. # noteworthy distinction from INDO @ noteworthy distinction from LB +Indo & A notable distinction from Chlorella +Indo $ A notable distinction from ESP +Indo.*

4. Discussion

A peptic ulcer (PU) is a prevalent condition affecting the gastrointestinal system in humans. It is known to occur when there is an imbalance between harmful and protective factors in the digestive tract. Previous studies have indicated that around 25% of gastric ulcer cases result from the use of NSAIDs, so their prevention and management are considered very important challenges [18].

Indomethacin has a documented higher risk of stomach ulcers than other commonly used NSAIDs. To cause gastric ulcers, indomethacin was chosen for this experiment even though the usage of recently developed NSAIDs is growing daily. This is because indomethacin is still widely used in rural areas because it is inexpensive. Moreover, it's one of the most popular NSAIDs for producing stomach ulcers in experiments [19].

The current treatment of gastric ulcers with antisecretory drugs, such as PPI and H2 antagonists, as long-term treatment, is associated with many side effects [20]. So, it was challenging to find other natural treatments to avoid these side effects. Several studies have been conducted to determine the physiological and biochemical roles of chlorella, including enhancing immunological function, preventing ulcers, acting as an antidiabetic, and treating dyslipidemia [21].

By preventing gastrointestinal illnesses, probiotics have long been known to strengthen human immunity. As a result, they work by producing antimicrobial chemicals, enhancing the function of the intestinal barrier, and immunomodulating [22]. All pre-treated groups in this study showed a substantial decrease in ulcer index as compared to the Indomethacin (INDO) group. The reversal of INDO's inhibitory action on PGE_2 production or the rebalancing of aggressive and protective gastric factors could account for this decrease [23]. Mucosal depth measurements in the Indo group were significantly lower than those in the control and other pre-treated groups.

The ESP +INDO and LB +INDO groups did not differ significantly from the control. Mucosal depth significantly increased in the Chlorella +INDO group when compared to the control and other pre-treated groups; this was consistent with the findings of previous studies [24, 25]. Hematoxylin and eosin-stained sections of the INDO group revealed several deep erosions, a malformed fundic gland architecture, and a loss of surface columnar mucus-secreting cells, together with blood vessel congestion and RBC extravasation.

The same results were found when these findings were compared to those of Emami et al. (2017) [26]. Wang et al. (2018) demonstrated how NSAIDs interfere with prostaglandin formation by inhibiting the COX enzymes, COX-1 and COX-2, which is the main cause of NSAID-induced

stomach ulcers [27]. As concurred with Patrick et al. (2018), who reported similar observations, the glandular mucosa displayed multifocal ulcerative patches, which were typified by an accumulation of hemosiderin pigment and necrotic tissue as well as desquamation of the epithelial lining coupled with hemorrhages [28]. Another study showed that one of the most harmful effects of NSAIDs on the digestive system is the acceleration of tissue oxidation [29]. Additionally, the generation of lipid peroxidation, superoxide radicals, and protein oxidation is accelerated by oxidative damage, causing erosion, bleeding, and ulcers in the stomach mucosa. Apoptosis was also evident in parietal cells. These findings were in line with those of Kang et al. (2017), who noted decreased nuclei and apparent cytoplasmic vacuolation as indicators of parietal cell degeneration [30]. One possible explanation for these vacuolations is the release of lysosomal enzymes that accumulate in parietal cells and cause the permeability of the cellular membrane to be disrupted. This is caused by oxygen-free radicals, which allow water and electrolytes to enter the cells more easily. This causes the cells to enlarge and organelles to break down, resulting in cytoplasmic vacuolation [31].

Moreover, the fundic glands' basal regions displayed apoptotic peptic cells. In accordance with those results of Abdel-Tawab et al. (2020) [32], who revealed that changes in the peptic cells as a result of lipase and pepsin's strong activity on the mucosal lining [32. 33].

Regarding the ESP +INDO group, examining the stomach's fundic sections revealed that the gastric mucosa's general architecture had been restored; however, there were still some places that had erosions. Some parietal cells had small nuclei which are darkly stained and had vacuolated cytoplasm. These findings were in line with Rani et al. (2018), who revealed the same observations [14]. There are several possible explanations for the gastro-protective benefits of ESP, including both acid-dependent and acid-independent systems. A previous study found that esomeprazole offers a protective effect through suppression of stomach acid production [34]. While another study revealed that there was a free radical scavenging property associated with esomeprazole and another PPI [1]. This was accomplished by increasing the expression and activity of the cytoprotective protein HO-1 in stomach epithelial and endothelial

cells. In the Chlorella +INDO group, the stomach mucosa showed a considerable protective effect. When the stomach's fundic sections were examined, the gastric mucosa's typical architecture was restored, and the pits were somewhat widened. Congested blood vessels were observed in the lamina propria. Fundic glands were restored, but some parietal and peptic cells showed small nuclei which are darkly stained and had vacuolated cytoplasm. These results were in the same line with Mohammed Ali et al. (2020), who explained this protective effect by the flavonoid content of chlorella, which has a strong anti-ulcerogenic action [35]. The flavonoids quercetin, rutin, and kaempferol caused suppression of stomach electrogenicity. Also, this protection is mediated by a significant inhibition of platelet-activating factor with a subsequent decrease in the secretion of histamine. Furthermore, another study demonstrated that quercetin promotes local prostaglandin production, reduces the secretion of histamine from gastric mast cells, and blocks the H+/K+ proton pump, resulting in a reduced amount of secreted gastric acid. Lactobacillus acidophilus was used in the current experiment to find out whether it has any protective role against INDO-induced gastric ulcers. Probiotics have been an increasingly popular therapy option in recent years, especially for the prevention of gastrointestinal tract disorders [37].

In the present work, when comparing pre-treated groups, the LB+INDO group showed better gastroprotective benefits. The architecture of the gastric glands returned to almost normal. Mucus neck cells and surface mucus cells appeared to be normal, as well as the majority of the parietal cells with acidophilic cytoplasm. Examination of the majority of gland bases revealed restoration of the normal morphology of parietal and peptic cells. This was in line with Mousa et al. (2023), who found comparable outcomes [38]. The study of Gwee et al. (2018) could support the earlier findings by indicating that lactate may have several gastroprotective benefits, including anti-inflammatory and anti-apoptotic qualities, in addition to preserving the integrity of the stomach mucosal barrier [39]. According to earlier research, lactate may lessen local inflammation by lowering the amounts of IL-1β, TNF-α, IL-1α, and IL-6 in stomach tissue [40, 41].

According to a previous study, Bcl-2 is one of the main elements influencing cell death [42]. Important members of the Bcl-2

family, the Bcl-2 and Bax proteins, are crucial for determining how long a cell has been around. They found that Bcl-2 inhibits apoptosis, whereas Bax causes it [43]. Therefore, a mismatch between the expression of the anti-apoptotic Bcl-2 family members and the apoptotic Bax proteins causes apoptosis.

Accordingly, increased Bcl-2 expression and decreased Bax expression may be responsible for the probiotic's anti-apoptotic activity in this investigation. This was in line with Mandi et al. (2019), who verified probiotics' anti-apoptotic properties [41].

In PAS-stained sections of the INDO group, there was weak PAS reactivity in the eroded surface epithelium, while the ulcerated areas had a total loss of PAS reaction. The INDO group's decreased stomach mucosal glycoprotein content was consistent with [44].

Different studies explained that the topical irritant properties of NSAIDs can decrease the stomach's mucus gel layer's hydrophobicity, which is a major defense against acid-induced stomach injury [45-47]. They proposed that these alterations resulted from reduced PG synthesis and mucus production due to injury to the mucus neck cells and surface epithelial cells. This reduces the capacity of pepsin and HCL to diffuse from the lumen into the mucosa. Acid and pepsin back-diffusion into the tissues causes further release of these substances, which in turn reduces stomach motility and mucosal blood flow.

Comparing the ESP+INDO group to the INDO group, it was found that the area percentage of the PAS reaction had significantly increased. This outcome was consistent with that of Tayeby et al. (2017), who found that PPI medication preserved the glycoproteins of the stomach mucosa [48]. The present result was likewise in agreement with Sengul et al. (2019), who discovered that the administration of PPI considerably raised the PAS reaction intensity of the mucosa's surface mucus and mucus neck cells [45].

Proton pump inhibitor treatment retained the stomach mucosal glycoprotein by its impact on raising the stomach NO level, which lowers the secretion of gastric acid and raises mucus secretion [49].

It was found that the Chlorella +INDO group's area percentage of PAS response was significantly greater than that

of the INDO and ESO+INDO groups. This result was consistent with Alese et al. (2018), who revealed improvement in mucus secretion [50]. The gastroprotective effects of microalgae are possibly due to their relatively high antioxidant capacity, as it has high phenolic acids and flavonoid content, leading to increased mucus secretion and substantially reduced plasma gastrin levels [3]. Antioxidants and flavonoids are abundant in chlorella. In addition to decreasing lipid peroxidation, flavonoids improve DNA synthesis, which prolongs the lifetime of collagen fibrils. Additionally, essential fatty acids can also be found in abundance in chlorella. It was established that eicosapentaenoic acid (EPA), alpha-linolenic acid, and gamma-linoleic acid (GLA) promote cell migration toward the injured sites and can enhance re-epithelialization [7].

The area percentage of PAS reaction increased significantly when the LB+INDO group was compared to the INDO group and other pretreatment groups. which concurred with the similar conclusions in previous studies [51-53], which claimed that probiotics also significantly affect the integrity of the stomach mucosal barrier by enhancing mucus secretion, preventing apoptosis, and encouraging cell proliferation. Consuming probiotics raised the expression of the gene for inducible nitric oxide synthase (iNOS), according to experimental studies on the subject [54].

When compared to all other groups, the Indomethacin (INDO) group exhibited a significant increase in area % in terms of NF-κB immunoreactivity.

These findings align with the study a previous study, which reported that INDO markedly upregulated NF-κB expression by approximately 101% relative to the control group [55]. Similarly, a previous study indicated that NSAIDs cause reactive oxygen species (ROS) generation and inflammatory reactions by impairing the protective phospholipid barrier of the stomach and interfering with oxidative phosphorylation in mitochondria [56]. The mechanism of this process, as indicated previously, is one of the key processes is oxidative stress and NF-κB production via the Nrf2/HO-1 pathway [57]. By elevating inflammatory cytokines, decreased Nrf2 expression worsens inflammation and raises the production of ROS.

In response to oxidative stress, NF-κB is activated with subsequent

downregulation of Nrf2 expression, which raises ROS and inflammatory cytokines. This, in turn, increases inflammation. In the same line, one of the possible mechanisms was the elevated phosphorylation of the MAPK cascade in ulcerated stomach tissue, including that of JNK, ERK, and p38. In the end, the phosphorylated MAPK cascade amplifies the activation of IκB-α, which triggers the NF-κB activity [58]. In contrast to the INDO group, there was a notable drop in the area percentage of NF-κB immunoreaction in the ESP-INDO group.

This mechanism was validated in a previous study, which showed that the inhibition of proinflammatory proteins, including TNF-α, interleukins (such as IL-1β and IL-6), nitric oxide synthase, and proteins of cell adhesion molecule 1, was connected to the anti-inflammatory activity of esomeprazole [59].

The Chlorella +INDO group showed a significantly lower area percentage of NF-κB immunoreaction than the INDO and ESO+INDO groups. This result was reported before, where the same observation was made [60, 61]. Furthermore, certain phenolics, including gallates, which are strong TNF-α inhibitors, may be responsible for *Chlorella vulgaris*'s anti-inflammatory qualities. Among other compounds, triterpenoids can reduce the expression of mediators of inflammation.

Additionally, it has been shown that ergosterol and peroxide-derived ergosterol from Chlorella vulgaris reduce pro-inflammatory cytokines [62]. NF-κB is strategically located at the intersection of inflammation and oxidative stress. It has been suggested that ROS could play a significant role as a secondary mediator in NF-κB activation in response to different stimuli. The Nrf2/HO-1-dependent pathway to avoid cellular damage was successfully initiated by *Chlorella vulgaris* treatment, as evidenced by the significant increase in Nrf-2 and HO-1 expression [63, 64]. The area percentage of NF-κB reaction was significantly lower in the LB+INDO group than in the INDO group and other pretreatment groups. That aligned with Ofusori et al. (2020) [65]. The prior findings may be explained by raising the levels of the anti-inflammatory cytokine IL-10 and lowering the levels of various pro-inflammatory cytokines in the serum, according to Fang et al. (2023), who discovered a protective effect of Lactobacillus acidophilus in ulcerative colitis [66].

5. Conclusion

As a pre-exposure prophylactic against Indomethacin (INDO)-induced stomach ulcers in rats, the results of this study show that oral treatment of Lactobacillus acidophilus and Chlorella offers considerable gastroprotection. Their protective benefits outweighed those of the standard esomeprazole medication. Compared to Chlorella, Lactobacillus acidophilus is more effective. Both medicines' positive effects seem to be partly explained by their capacity to maintain gastroprotective systems and reduce oxidative stress. Therefore, Chlorella and Lactobacillus acidophilus could be useful therapeutic agents for preserving the delicate equilibrium between defensive and aggressive stomach forces.

AI deceleration: Not applicable

Ethical approval: Ethical committee No.102 on 15 January 2023, Faculty of Medicine, Fayoum University, approved this study.

Funding: There are no funds for this research.

Conflicts of Interest: None of the authors of the current study discloses any conflicts of interest.

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