"The effect of Probiotics on Aspirin induced Gastric Mucosal Lesion (Gastric ulcer) in comparison to Pantoprazole in male Wistar rats - An Experimental Study"

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ABBREVIATIONS

PUD: Peptic ulcer disease
IBD : Inflammatory bowel disease
GIT : Gastrointestinal Tract
5-ASA : 5-Aminosalicylic Acid
TNF-α : Tumor necrosis factor – α
ROS : Reactive Oxygen Species
RNS : Reactive Nitrogen Species
NF-κB : Nuclear factor kappa-light-chain-enhancer of activated B cell
IL : Interleukin
IP : Intra Peritoneal
EDTA : Ethylene Diamine Tetra Acetic acid
TGF : Tumor Like Growth Factor
NSAIDs : Non-steroidal anti-inflammatory drugs
CS : Corticosteroids
GC : Glucocorticoids
IFNγ : Interferon gamma
VCAM : vascular cell adhesion molecule
KO : Gene Knockout
PG-PS : Peptidoglycan-Polysaccharide
STAT : Signal Transduction and Activator of Transcription
TCR : T Cell Receptor
RCT : Randomized Controlled Trial
DSS : Dextran Sulphate Sodium
DNA : Deoxyribose Nucleic Acid
RNA : Ribonucleic Acid
KDa : Kilo Dalton
IAEC : Institutional animal ethics committee
CCSEA : Committee for the control and supervision of experiments on animals
DAI : Disease activity index
MDA : Malonaldehyde
CDNA : Complementary DNA
PCR : Polymerase Chain Reaction
RT-PCR : Real Time - Polymerase Chain reaction
GAPDH : Glyceraldehyde 3 Phosphate Dehydrogenase
v/v : Volume by Volume

ABTRACT

INTRODUCTION :

Aspirin, a Globally used NSAIDS drug , is meant for its analgesic, antipyretic, and antiplatelet benefits . However, its long-term use is associated with gastric mucosal lesion and ulcer formation, primarily due to cyclooxygenase-1 inhibition, which decrease protective PGs synthesis. The outcome because of its decreased in mucus and bicarbonate release, increased gastric acid formation, oxidative stress, and inflammation, helpful in mucosal erosion and improving ulcer healing.

Proton pump inhibitors , like pantoprazole, are the routine treatment for Non – steroidal anti-inflammatory drugs-induced gastric leasion, as they inhibit gastric acid release and improving ulcer healing. However, long term Proton pump inhibitors use has been involved with gut microbiota alteration , rebound increased acid , and disturbed nutrient absorption. Due to these apprehension, probiotics have emerged as a potential adjunctive therapy for gastric ulcer treatment , given their ability to modify gut microbiota, improving mucosal safty and apply, anti-inflammatory impact .

The study intended to contrast the gastrointestinal safety effects of probiotics and pantoprazole in an aspirin-treated gastric ulcer screening in male Wistar rats by evaluating inflammatory cytokine levels, histopathological changes, and mucosal integrity.

OBJECTIVES

The primary goal of the study are:

- Estimate probiotic effect on gastric mucosal injury induced by treatment of aspirin in male Wistar rats.
- Differentiate the effects of probiotics with Pantoprazole for gastroprotective , a widely used PPIs , escape from aspirin-treated mucosal damage.

Secondary Objective

Investigate how probiotics affect the value infllammatory cytokines, particularly TNF-alpha, interleukin -2, and interleukin -4.

METHODOLOGY :

This experimental study was done on male Wistar rats (150–170 g), which were grouped into four and each groups consist (n = six (6) per group):

1. Normal control group (treated with saline).
2. Aspirin control group (treated aspirin-induced ulceration without treatment).
3. Pantoprazole-treated group (received aspirin + pantoprazole at 7.2 mg/kg).
4. Probiotic-treated group (received aspirin + probiotic blend containing *Lactobaciillus acidophiilus, L.. plantarumm, L. rhammnosus, & L. caseii at 90 mg/kilo).*

Aspirin (500 mg/kg) was given orally on Day 15 to induce gastric ulcers after a 14-day pre-treatment phase.

Blood samples were gathered for inflammatory cytokine evaluation (TNF-alpha , interlukin-2, and interlukin-4 levels).

Gastric tissue samples were evaluated for histopathological alteration , may add epithelial rupture , necrosis, inflammatory cytokines , and glandular rupture.

Statistical analysis was done with the help of ANOVA followed by Tukeey's post hocs investigation to contrast management effects, with $p \leq 00.05$ statistically significants..

RESULTS :

Management with Aspirin significantly elevate TNF-alpha and interleukin -2 levels where as reduced IL-4 levels, shows severe inflammation and impaired mucosal healing.

Pantoprazole treatment markedly reduced TNF-alpha and interleukin -2 levels, increased interleukin -4 expression, and improved mucosal integrity by suppressing acid secretion.

Probiotic-treated rats manifest notable decrease in inflammatory level , improved mucus secretion, and enhanced mucosal defense. However, their anti-inflammatory effect was moderate compared to pantoprazole.

Histopathological evaluation showed that pantoprazole was more effective in decrease epithelial disruption , necrosis, and inflammatory infiltration, while probiotics show a slower, microbiota-dependent protective effect.

A combined approach using probiotics with Proton pump inhibitors could provide more comprehensive management plan by equating immediate ulcer healing with long-term gastric health.

Conclusion:

This study highlights aspirin-induced gastric ulcers, causing mucosal damage, & inflammation. Pantoprazole effectively promotes acute ulcer healing by inhibiting acid secretion and reducing inflammation but raises concerns with long-term use. Probiotics (Lactobacillus strains) show gastroprotective potential by modulating inflammation and enhancing mucosal defense. While not a substitute for PPIs in acute

cases, they offer promise for long-term gastric health. Future research should focus on clinical trials and strain-specific formulations to optimize probiotic use alongside PPIs for a safer, integrative ulcer treatment approach.

KEYWORDS:

Aspirin-induced gastric ulcer, Proton Pump Inhibitors (PPIs), Pantoprazole, Probiotics, NSAID-induced ulcer, Gut microbiota, Lactobacillus species, Inflammation, Gastric mucosal protection.

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INTRODUCTION

Chronic lesions in the gastrointestinal (GI) tract known as peptic ulcers develop when the protective mucosal layer is compromised. This deterioration results in well-defined ulcers that pierce the muscularis mucosae by allowing gastric acid to reach the underlying tissues and

digesting enzymes. Chronic & acute inflammatory cells usually surround these lesions Stomach peptic ulcers can develop, duodenum, or esophagus, but they most commonly form in the duodenum. Peptic ulcers develop when there is an imbalance between detrimental elements and defense systems, such as mucus and bicarbonate secretion and sufficient mucosal blood flow, including gastric acid, pepsin, and external influences like *H.pylori* infections, smoking, drinking alcohol, and using nonsteroidal anti-inflammatory drugs (NSAIDs). Ulcers and mucosal damage result from this balance being upset [(1).]

The development of ulcers is significantly influenced by lifestyle variables. diets that include a lot of spicy foods or that cause the body to produce too much acid might make symptoms worse. Furthermore, via impairing mucosal blood and lowering bicarbonate secretion

flow, alcohol use and smoking both weaken mucosal defence [(2).]

Chronic stress and a sedentary lifestyle also raise the risk of ulcer development by increasing stomach acid secretion via neurohormonal pathways. There is strong evidence linking NSAID usage, especially aspirin, to peptic ulcer disease recorded (3).

The cyclooxygenase enzyme, especially COX-1, which is essential for the stomach lining's synthesis of protective prostaglandins, is inhibited by aspirin. Enhancing mucosal blood flow and encouraging the release of mucus and bicarbonate, these prostaglandins

support mucosal integrity. The gastrointestinal lining becomes weaker when their synthesis declines, increasing its vulnerability to damage by stomach acid and pepsin [4].

Numerous adverse consequences, including peptic ulcer disease, gastritis, and even potentially lethal gastrointestinal bleeding, are associated with long-term aspirin use . Aspirin-induced ulcers can show clinically in a variety of ways, from no symptoms at all to excruciating stomach discomfort. Discomfort, hematemesis (blood vomiting), melaena (black, tarry stools), and iron deficiency anemia from persistent blood loss are further potential symptoms [5].

Due to increased stomach acid secretion after meals, the discomfort, which is often cantered in the epigastric area, tends to get worse. These possible side effects highlight the necessity of practical methods to lessen NSAIDs' detrimental effects on the stomach mucosa [6].

Probiotics, which are living microorganisms, have attracted substantial interest recently due to their ability to support and restore intestinal health when ingested in appropriate numbers [7].

Among these beneficial microorganisms are several bacterial strains with distinct modes of action, such as Lactobacillus, Bifidobacterium, and Saccharomyces. Probiotics not only promote gut health but also improve intestinal barrier function, lower inflammation, and regulate the immune system [8].

Probiotics have been widely examined for their involvement in controlling gastrointestinal illnesses, such as irritable bowel syndrome and diarrhoea linked to antibiotics [9].

They have several beneficial benefits, including as boosting mucosal barrier function, controlling immunological activity, competing with harmful bacteria, and generating antimicrobial chemicals [10].

Certain strains have shown antioxidant qualities that may help reduce oxidative stress caused by reactive oxygen species, particularly those from the Lactobacillus and Bifidobacterium families, which is one of the main causes of stomach damage brought on by NSAIDs [11].

Probiotics provide a potentially effective treatment option for aspirin-induced stomach damage. By promoting producing mucus and strengthening tight connections in the epithelium, they improve barrier function and lessen the influx of dangerous substances, supporting mucosal defense [12].

Furthermore, by decreasing the synthesis of NSAIDs need pro-inflammatory cytokines include interleukin-1 beta, interleukin-6, and tumor necrosis factor - alpha. -induced harm to the stomach—probiotics aid in the regulation of inflammation [13].

Furthermore, by promoting angiogenesis in the stomach lining and inducing the production of growth factors, probiotics may aid in mucosal repair [14].

Probiotics may help prevent stomach ulcers brought on by aspirin, although their precise function is yet not well understood. The majority of current research has focused on how well they work for ailments includes inflammatory bowel disease (IBD) and diarrhea linked to antibiotics . By strengthening mucosal protection and reducing inflammation, probiotics may lessen the harm that NSAIDs cause to the stomach , according to preliminary animal research. Nevertheless, there are currently few thorough clinical studies evaluating their effectiveness in human individuals [15]. This information gap emphasizes the need for more research on probiotics' potential as a preventative measure against stomach ulcers brought on by NSAIDs. By assessing the preventive benefits of a particular probiotic mix, which includes strains that have been freeze-dried or cryopreserved, against aspirin-induced stomach ulcers, this study seeks to close that gap. The study will concentrate on evaluating inflammatory marker levels, and mucosal

damage. The goal of this study is to provide focous on the possible significance Using probiotics as an adjuvant treatment to reduce stomach damage caused by NSAIDs, considering the widespread use of NSAIDs and the serious consequences of peptic ulcer disease.

The study's objectives are:

Following are the objectives of this experimental study:

Primary objective:

• To assess the effect probiotics male Wistar aspirin-induced ulcers or lesions of the stomach mucosa.

• To evaluate the gastroprotective effectiveness of probiotic in reducing aspirin-induced mucosal damage in comparison to pantoprazol, a common proton pump inhibitor.

Secondary objective:

- To determine the Probiotics' impact on inflammatory cytokines, specifically TNF-alpha, IL-2, and IL-4.

REVIEW OF LITERATURE

Peptic Ulcer Disease (PUD)

A prevalent gastrointestinal ailment called peptic ulcer disease (PUD) is brought on by an imbalance between beneficial and detrimental gastric components. It is marked by erosion of the stomach or duodenum's mucosa. It still presents a significant global health concern, resulting in significant morbidity and higher global healthcare costs [16].

Millions of people worldwide suffer from peptic ulcer disease (PUD), which is thought to affect 5–10% of the population. Geographical variations in its prevalence are caused by a number of variables, including the frequency of *H. pylori* infections, the utilization of nonsteroidal anti-inflammatory medicines , and different lifestyle factors [17].

. In Western nations, the incidence of PUD has decreased, largely due to better sanitation and the extensive application of H. pylori eradication therapies. Conversely, in developing countries, the disease remains prevalent, primarily due to the continued presence of H. pylorii infections and the rising use of nonsteroidal anti- inflammatory drugs [18]. Peptic ulcer disease tends to be more common in men than in women, with duodenal ulcers occurring more frequently than gastric ulcers. Older adults are especially at risk, largely due to the extensive use of aspirin and other NSAIDs for the prevention of cardiovascular diseases [19].Hospital admissions for peptic ulcers remain substantial, with complications such as bleeding, perforation, and obstruction significantly increasing mortality, particularly in elderly patients and those with underlying health conditions [20]. The development of PUD is influenced by multiple factors, with the main offenders being H. pylori infection and NSAID usage; further contributory variables include

excessive production of stomach acid, smoking, alcohol intake, and stress-induced mucosal injury [21] . The stomach mucosa is home to the Gram-negative bacteria *H. pylori* triggering chronic inflammation and stimulating excess acid production. It releases virulence factors such as urease, cytotoxin-associated gene A (CagA), and vacuolating cytotoxin A (VacA), which compromise the mucosal barrier and contribute to ulcer formation [22]. Ulcers associated with H. pylori are predominantly found in the duodenum and can result in major issues including bleeding or perforation if treatment is not received [23] . NSAIDs, including aspirin, are a major cause of PUD, particularly in patients requiring long-term use for cardiovascular and inflammatory conditions. These drugs inhibit cyclooxygenase -1, decreasing PGs synthesis, which alters mucus and bicarbonate production, weakens mucosal blood circulation, and increases acid secretion, leading to ulcer formation [24]. Aspirin-induced ulcers are often asymptomatic until complications such as gastrointestinal bleeding occur [25]. Excessive gastric acid and pepsin secretion contribute to ulcer formation by overwhelming mucosal defense mechanisms. Conditions such as Zollinger-Ellison syndrome, which causes hypersecretion of gastric acid due to gastrin-producing tumors, significantly increase PUD risk [26]. Smoking and alcohol consumption increase ulcer risk by impairing mucosal healing, reducing bicarbonate secretion, and promoting gastric acid production. Stress-induced ulcers, commonly observed in critically ill patients, are linked to reduced gastric perfusion and mucosal ischemia [27]. Genetic predisposition significantly influences susceptibility to peptic ulcer disease (PUD), especially in those who have a family history of the illness.. Additionally, environmental elements including inadequate personal hygiene and socioeconomic status contribute to the transmission of *Helicobacter pylori*, impacting disease prevalence. The complex interplay of genetic and environmental factors in PUD pathogenesis underscores the need for effective

management strategies. In this context, comparing probiotics and pantoprazole's effects on Wistar rats' aspirin-induced gastric mucosal lesions provides valuable insights into potential alternative or adjunct therapies for preventing NSAID-related gastric damage (28).

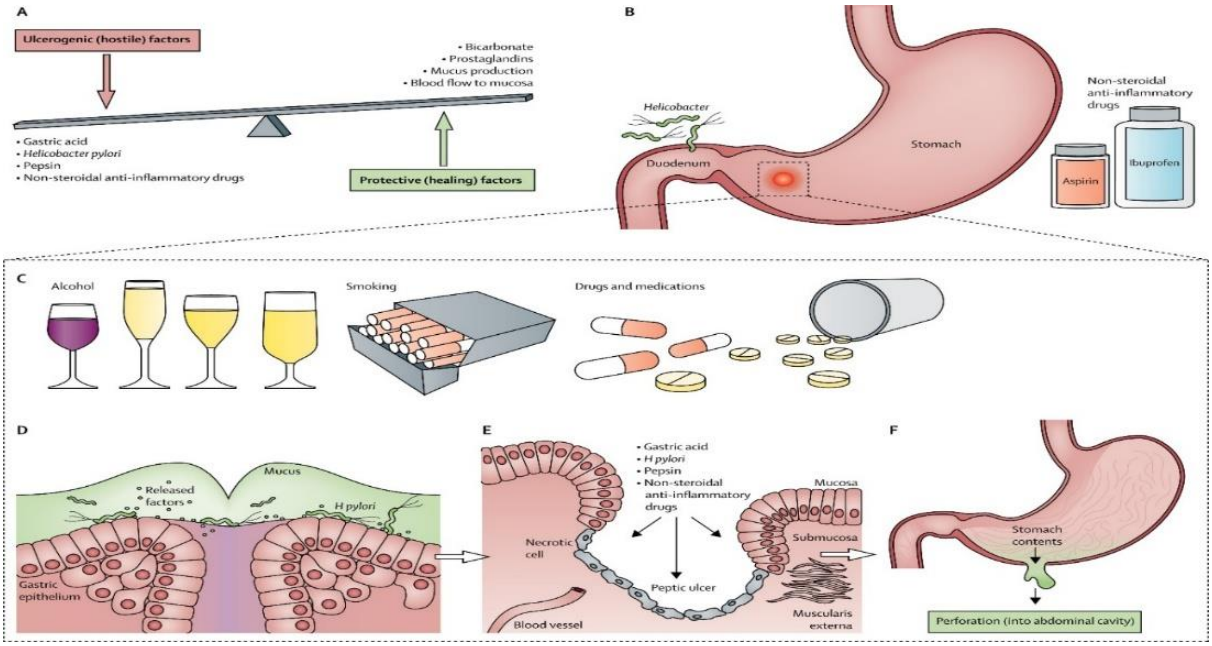


Figure 1 – Etiology and factor affecting Peptic ulcers disease.[118]

Pathophysiology:

Peptic ulcer disease (PUD) is brought on by an imbalance between the stomach mucosa's defense mechanisms and harmful elements such acid, pepsin, H. pylori infection, and the use of nonsteroidal anti-inflammatory drugs (NSAIDs) . A complex defensive system that involves synthesis of mucus and bicarbonate, continuous regeneration of epithelial cells, and adequate circulation of mucosal blood protects the stomach mucosa against harm caused by digesting enzymes and gastric acid. When these defenses are compromised, ulcers and mucosal damage result [(29).]

One of the leading causes of Peptic ulcer disease is an H. pylori infection is a major contributing factor to ulcer formation through various mechanisms. It colonizes the gastric mucosa and secretes pathogenicity factors such as vacuolating cytotoxin A (VacA) and cytotoxin-associated gene A (CagA), which disrupt the protective mucosal barrier. which trigger an inflammatory response, leading to increased gastric acid release and mucosal injury. Additionally, *H. pylorii* alters production of somatostatin, a hormone that inhibits acid secretion, further promoting an acidic environment that predisposes the gastric lining to damage [(30).] Chronic inflammation aggravated by *H. pylorii* also weakens the mucosal barrier by impairing epithelial cell regeneration and increasing oxidative stress, further exacerbating tissue injury [(31)] NSAID-induced ulcers are primarily caused by cyclooxygenase-1 (COX-1) suppression , an enzyme necessary for the synthesis of prostaglandins. Because they promote mucus and bicarbonate production, act as a barrier against stomach acid, improve mucosal blood circulation, and aid in epithelial healing, prostaglandins are essential for maintaining the integrity of the stomach mucosa . The stomach is more susceptible to acid-related injury because the decrease in prostaglandin synthesis impairs mucosal defenses. Furthermore, by increasing mucosal permeability, causing mitochondrial dysfunction, and inducing apoptosis, NSAIDs directly harm stomach epithelial cells, all of which lead to the development of ulcers [(32).]

Hypersecretion of gastric acid is another key factor in PUD pathogenesis. Parietal cells release gastric acid in reaction to histamine, acetylcholine, and gastrin, plays a central role in ulcer formation by breaking down mucosal proteins and impairing barrier function. Conditions such as Zollinger-Ellison syndrome, which leads to excessive gastrin secretion and hyperchlorhydria, significantly increase the risk of peptic ulcer formation due to prolonged acid exposure [(33).]

Oxidative stress is a key contributor to ulcer formation, as excessive Lipid peroxidation, DNA damage, and stomach epithelial cell death are all brought on by reactive oxygen species (ROS).

Neutrophils more aggravate this process by releasing inflammatory mediators and proteolytic enzymes, compromising mucosal integrity and delaying the healing process [34]. Moreover, cytokines that promote inflammation, such as interleukin-1 beta and tumor necrosis factor-alpha (TNF-α) amplify inflammation, intensifying tissue injury and prolonging ulcer persistence [35].

Ulcer healing may be delayed when factors like chronic *H. pylorii* infections, extended NSAIDs usage, or ongoing oxidative stress disrupt epithelial regeneration, angiogenesis, and extracellular matrix remodelling. Important growth factors, such as epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF), are crucial in facilitating mucosal repair by promoting tissue regeneration and wound healing, but their activity may be impaired in the presence of chronic inflammation and oxidative damage, increasing the risk of recurrent ulceration [36].

Additional factors such as smoking and alcohol consumption further contribute to PUD by impairing mucosal blood circulation , reducing bicarbonate release , and increasing gastric acid Formation , thereby delaying ulcer healing and worsening disease severity [37]. Moreover, genetic predisposition and environmental factors, including dietary habits and socioeconomic conditions, affect an individual's at risk to PUD, with studies indicating the genetic polymorphisms affecting cytokine production and mucosal defense mechanisms may play a role in ulcer development [38].

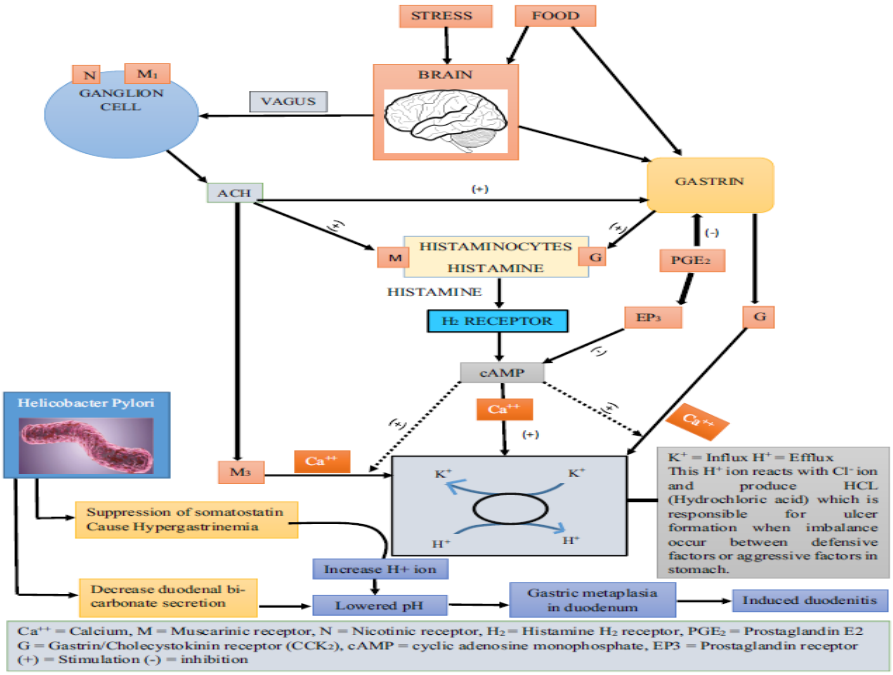


Figure. 2 – Secretion of gastric acid and pathophysiology of peptic ulcer disease[118]

Clinical course of peptic ulcer disease:

Intermittent epigastric discomfort that gets worse after eating is frequently the early symptom of gastric ulcer disease, however the clinical course varies. Other symptoms might be bloating, nausea, and an early sense of fullness, but some people don't have any symptoms until problems arise. Ulcers can cause serious consequences such gastrointestinal bleeding and penetration if they are not managed [(39).]

Patients may develop melena, hematemesis, or even peritonitis in extreme situations. Most ulcers go away in a few weeks with the right care, This comprises drugs for the eradication of H. pylori and proton pump inhibitors (PPIs). Recurrence is still a worry, though, especially in people who have persistent risk factors. Additionally, persistent ulcers may arouse suspicions of cancer, requiring endoscopic examination [(40).]

Diagnosis of Peptic Ulcer Disease

Clinical evaluations , Laboratory tests, & endoscopic examinations, are the main methods used to evaluate stomach lesions. Patients frequently complain of bloating, nausea, and epigastric discomfort, and these symptoms may get worse after giving birth. A thorough medical history is crucial, especially when it comes to the use of NSAIDs and risk factors including alcohol, smoking, and H. pylori infection . Upper gastrointestinal Because it allows for direct sight of lesions, evaluation of their size and depth, and the collection of tissue samples to rule out malignancy, endoscopy is still the gold standard for diagnosing stomach ulcers [41].

It is possible to distinguish between benign and malignant ulcers by histopathologically analyzing biopsy samples. Moreover, the underlying cause can be identified by Noninvasive methods of diagnosing H. pylori, include the urea breath test, fecal antigen collection, fecal antigen collection, and blood assays [42]. While tests such as complete blood sample (CBC) can detect anemia caused by chronic blood loss, fecal occult blood testing helps detect gastrointestinal bleeding. When endoscopy is not an option, barium meal radiography is a rare method that can be used. Computed tomography (CT) scans and other sophisticated imaging modalities are normally kept for diagnosing abnormalities like stomach outlet obstruction or perforation.

Management and Available Drugs for Peptic ulcer disease:

Peptic ulcers disease primarily results from the inhibition of cyclooxygenase-1, leading to reduce the PGs synthesis. By decreasing mucosal blood flow, increasing the formation of stomach acid, and affecting mucus and bicarbonate secretion, this lowering undermines the gastric mucosal barrier. preventing ulcer recurrence, encouraging

mucosal healing, and lowering complications are the main goals of management techniques [43].

Treatment includes lifestyle modifications, pharmacological interventions, surgical options in refractory cases and emerging therapeutic strategies.

Lifestyle Modifications

Specific lifestyle changes contribute significantly to ulcer prevention and healing. Reducing alcohol consumption and quitting smoking are crucial because alcohol damages mucosal integrity and increases irritation, while smoking slows the healing of ulcers and increases Helicobacter pylori (H. pylori) virulence. A diet high in fermented foods and fiber (e.g., yogurt, kimchi), and antioxidants (e.g., berries, green tea) supports gastric mucosal healing, whereas acidic, spicy, foods should be ristricted to prevent irritation. In patients needs prolong aspirin treatment, co-administration of proton pump inhibitors (PPIs) is recommended to reduce ulcer risk [44].

Pharmacological Management

The primary pharmacological strategies for peptic ulcers disease involve acid suppression, mucosal protection, and *H. pylori* eradication if necessary [45].

PPIs, or proton pump inhibitors

PPIs are the backbone of treatment since they act by permanently inhibiting the parietal cells' H^+/K^+ ATPase enzyme, which lowers stomach acid production and promotes ulcer repair (46). Common PPIs include omeprazole, pantoprazole, lansoprazole, and esomeprazole, typically administered at 40 mg/day for 1 – 2 moths. PPIs provide superior acid suppression and longer-lasting effects. However, prolonged use is

associated with potential risks, such as bacterial overgrowth, altered mineral absorption, and increased susceptibility to *Clostridium difficile* infections [47].

H_2 Receptor Antagonists and Antacids

H_2 receptors antagonist, including cimetidine & famotidine, inhibit histamine receptors in the stomach lining to reduce acid production. While less effective than PPIs, they serve as an alternative for patients intolerant to PPIs. Antacids, such as magnesium hydroxide and calcium carbonate, provide temporary symptomatic comfort by negotiate gastric acids but not help promote ulcer repair [48].

Prostaglandin Analogues (Misoprostol)

A synthetic prostaglandin E1 analogue called misoprostol promotes the release of mucus and bicarbonate, which improves mucosal protection. It is particularly beneficial for patients requiring continuous aspirin therapy. The recommended dosage is 200 mcg qid daily, but gastrointestinal adverse effects like diarrhoea limit its use [49].

***Helicobacter pylori* elimination management**

Antibiotic eradication therapy and acid suppression medication are required if an H. pylori infection is diagnosed. For seven to fourteen days, standard triple treatment consists of 500 mg of clarithromycin, 500 mg of amoxicillin or metronidazole, and a PPI (such as omeprazole 20 mg). In opposition to situations, a bismuth-based regimen added to triple treatment improves eradication successfully [50].

Available Treatments

peptic ulcers disease treatments primarily focus on acid suppression, mucosal protection, *H. pylori* eradication, and lifestyle adjustments. PPIs remain the most effective pharmacological option, while probiotics and antioxidants show promise in supporting mucosal healing.

Table 1 : Various medications are available in order to treat peptic ulcer illness, aiming to reduce symptoms, promote healing, and prevent complications.

Drug Class	**Examples**	**Mechanism of Action**	**Recommended Dosage**	**Key Considerations**
Proton Pump Inhibitors	Pan top , Rabeprazole	Strong acid suppression by irreversible blocks H^+/K^+ ATPase in parietal cells.	40 mg/day for 1 -2 months	Most effective for ulcer healing, reduces acid secretion significantly.
H_2 Receptor Antagonists	Ranitidine, Famotidine	Inhibit histamine receptors, reducing gastric acid production.	cimetidine: 150 mg twice daily, Famotidine: 20 mg BD daily	Less effective than PPIs but can be used as an alternative.
Antacids	Magnesium hydroxide, Aluminum hydroxide, Cal. carbonate	relieve symptoms and neutralize stomach acid.	Taken as needed	Provide temporary relief but do not heal ulcers.
Prostaglandin Analogue	Misoprostol	Increase mucosal	200 mcg QID daily	Can cause gastrointestinal

		protection by aggravating mucus and bicarbonate release .		Adverse effects like diarrhoea.
Helicobacter pylori Eradication Therapy	Clarithromycin, Amoxicillin, Metronidazole, PPIs (Triple/Quadruple Therapy)	Eradicates *H. pylori* infection, reducing ulcer recurrence.	Triple Therapy: 7–14 days	Required if *H. pylori* infection is present.
Probiotics	*Lactobacillus acidophilus*, *Lactobacillus rhamnosus*	Modulates gut microbiota, enhances mucus secretion, reduces *H. pylori* colonization.	Varies by formulation	Supports gut health and mucosal healing.
Antioxidants	Vitamin C, Vitamin E, Flavonoids (e.g., Quercetin)	Neutralizes oxidative stress, preventing mucosal damage.	Varies by formulation	May help in ulcer healing and reducing inflammation.

In refractory cases or those with severe complications, surgical intervention may be required to restore gastrointestinal function and prevent further severity [51].

Surgical Intervention

Surgical intervention is reserved for complicated cases, including persistent ulcers unresponsive to medical therapy, severe gastrointestinal bleeding, perforation, and gastric outlet obstruction. Surgical techniques include partial gastrectomy for recurring or malignant ulcers, gastrojejunostomy for blockage of the gastric outlet, and vagotomy with pyloroplasty to decrease acid secretion [52].

Emerging Therapeutic Approaches

Recent research has highlighted alternative treatment strategies that increase mucosal defense and promote ulcer repair .

Probiotics

Probiotics, particularly *L.bacillus* species, helpful to gastric tissue by restoring gut microbiota balance, promoting mucuss secretion, and reducing *H. pylori* colonization. Their mechanisms include inhibiting *H. pylorii* adhesion to gastric tissue, strengthening the mucosal barrier, and modulating immune responses. Studies indicate that probiotic supplementation alongside standard therapy improves *H. pylori* elimination rates and reduces antibiotic-associtate gastrointestinal adverse effects. Furthermore, probiotics mitigate oxidative stress and inflammation in the gastric mucosa, thereby accelerating ulcer healing [53].

Antioxidant Therapy

Oxidative stress is essential for stomach ulcers pathogenesis aggravated by aspirin, leading to lipid peroxidation, DNA rupture, and apoptosis of gastric tissue. Antioxidants, By scavenging free radicals, vitamins C, E, and flavonoids help prevent oxidative

damage, reducing inflammation, and promoting tissue repair. These compounds have demonstrated potential in supporting ulcer healing and protecting against aspirin-induced mucosal injury [54].

Brain – gut – skin axis hypothesis

The brain-gut-skin axis concept posits a bidirectional association network linking the CNS , gastrointestinal tract, and skin. This concept suggests that psychological stress can influence gut microbiota composition, leading to systemic inflammation that manifests in various skin conditions. Conversely, skin disorders can impact mental health, indicating a complex interplay among these systems [55,56]. Psychological strain may disturb the gut microbiota, causes enhance the intestinal penetrability ("leaky gut"). This permite microbial metabolites and toxins into the blood circulation, triggering systemic inflammation [57]. The translocation of microbial products into the bloodstream may trigger the immunological system, resulting in degree of pro-inflammatory marker secretion . These cytokines can affect skin homeostasis, potentially exacerbating conditions like acne, psoriasis, and rosacea [58]. Inflammatory responses can disrupt skin barrier function, leading to or worsening dermatological conditions. For instance, acne vulgaris has been associated with alterations in gut microbiota, and interventions targeting gut health have shown promise in alleviating symptoms [56,57]. The brain-gut-skin axis loop can be sustained by chronic skin diseases that have a detrimental impact on mental health by increasing stress, anxiety, or depression [59].

Probiotic – gut, health and wound healing

Probiotics acts a significant impact in gut health and general health, and wound healing by modulating the microbiota, enhancing immune function, and promoting tissue

regeneration. The microbiota in the gut is essential for digestive health, with probiotics aiding in restoring microbial balance, preventing dysbiosis, and reinforcing the gut barrier. certain strains,

such as *L. rhamnosus* and *Bifidobacterium breve*, have demonstrated effectiveness in reducing the signs and symptoms of gastrointestinal conditions such as inflammatory bowel disease and irritable bowel syndrome by modulating gut-associated immune responses and reducing inflammation [60]. Additionally, probiotics encourage the synthesis of butyrate and other short-chain fatty acids, which provide intestinal epithelial cells with energy and contribute to maintaining intestinal integrity [61]. A well-balanced gut microbiota supports systemic health by influencing metabolic functions, immune regulation, and even mental well-being.

Probiotics also having significant impact on wound repair through their capacity to lessen inflammation and oxidative stress while promoting tissue regeneration. Certain probiotic strains, including *Lactobacillus plantarum*, have been found to improve wound closure by enhancing collagen formation and modulating local immune responses [62]. The gut-skin axis emphasizes the impact of probiotics even more on skin health, as a well-balanced microbiota reduces systemic inflammation and supports skin barrier function, indirectly improving wound healing outcomes. Probiotics have also shown potential in post-surgical recovery by decreasing infection risks, enhancing immune responses, and reducing inflammatory complications in wounds, including diabetic ulcers and pressure sores [63].

Beyond gut and wound health, probiotics exert broad pleiotropic effects, including supporting immune function, reducing the risk of infections, and improving metabolic disorders. Their ability to modulate the immune system contributes to enhanced

resistance against pathogens, reducing the incidence of gastrointestinal and respiratory infections. Furthermore, emerging research suggests that probiotic supplementation may improve wound healing in patients with chronic inflammatory conditions by promoting beneficial microbial interactions and reducing excessive immune activation [64]. The extensive benefits of probiotics in gut health and tissue repair underscore their potential as a therapeutic intervention in both clinical and preventive healthcare settings.

Pleiotropic effect of probiotic strain in health and disease

Probiotic strains exert pleiotropic effects, influencing various physiological processes that contribute to both health maintenance and disease prevention. These beneficial microorganisms contribute significantly to gut microbiota regulation, strengthen immune responses, and exert systemic effects that influence various organ systems. Specific strains, such as *Lactobacillus plantarum* and *Bifidobacterium bifidum*, have been found to help mitigate symptoms of irritable bowel syndrome by promoting microbial balance and decreasing inflammation [65]. Additionally, *Faecalibacterium prausnitzii*, a key butyrate-producing bacterium, exhibits anti-inflammatory properties and is related with improved gut barrier action, potentially reducing the risk of conditions like Crohn's disease [66]. Probiotics also contribute to the prevention of antibiotic-associated diarrhea, with *Saccharomyces boulardii* and *Lactobacillus rhamnosus* demonstrating efficacy in maintaining intestinal health during and after antibiotic treatment [67].

Beyond gastrointestinal health, probiotics have shown promise in dermatological conditions. Their ability to modulate systemic inflammation and enhance skin barrier function makes them beneficial in managing skin disorders such as eczema and acne [68]. Certain strains have also been linked to respiratory health, decreasing the incidence and severity of infections through immune system modulation [69]. In women's health,

probiotics have demonstrated effectiveness in decreasing the recurrence of vaginal and UTI , underscoring their role in maintaining urogenital homeostasis [70]. Furthermore, upcoming trails suggests that probiotics may impact on liver disease management by decreasing inflammation and improving gut-liver axis function [71]. Some studies even highlight the potential benefits of probiotics in neuro health, as the gut microbiota influences neurotransmitter production, which may impact on situation like anxiety and psychological health [72]. Collectively, the pleiotropic effects of probiotic strains emphasize their therapeutic potential across a broad spectrum of health conditions.

Association between probiotics and wound healing, probiotics and inflammation:

Probiotics have gained attention for their potential role in modulating inflammation and enhancing wound healing through their effects on the gut microbiota, immune regulation, and tissue growth. Wound repair is a mixed biological condition involving inflammation, proliferation, and remodelling, and probiotics can influence these stages by modulating microbial populations lowering oxidative stress and strengthening immunological responses. Because fibroblast proliferation and collagen production are essential for tissue regeneration, several strains of *L.plantarum* and Bifidobacterium breve have been demonstrated to speed wound closure [73]. Additionally, probiotics contribute to wound healing by reducing the risk of infections. Their ability to enhance the skin's barrier function and inhibit pathogenic bacteria helps prevent wound-related complications, particularly in conditions like diabetic ulcers and post-surgical wounds [74].

The anti-inflammatory properties of probiotics are key to their beneficial effects in wound healing. Chronic wounds are often linked to excessive inflammation, which delays healing by increasing tissue damage and impeding the regeneration process. Probiotics regulate inflammatory pathways via increasing the release of cytokines that

reduce inflammation while inhibiting excessive activation of pro-inflammatory mediators, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) [75]. This immunomodulatory effect has been observed in both systemic and local inflammation, contributing to improved wound healing outcomes. Furthermore, probiotics enhance gut health, which impacts on systemic inflammation control. A well-balanced gut microbiota prevents intestinal permeability, reducing the translocation of bacterial endotoxins that can exacerbate systemic inflammation and impair tissue healing [76].

Probiotics have also demonstrated efficacy in reducing inflammation-associated conditions beyond wound healing, including inflammatory bowel disease, arthritis, and metabolic disorders. The gut microbiota impacts on immune homeostasis, and probiotic supplementation has been associated with reduced markers of chronic inflammation in these conditions. For example, *Lactobacillus rhamnosus* and *Bifidobacterium longum* have been brought to the attention of decrease inflammatory responses Among those who have Crohn's illness and ulcerative coliitis by restoring microbial balance and enhancing mucosal barrier function [77]. Similarly, in arthritis models, probiotics have shown the ability to decrease joint inflammation by influencing gut-derived immune pathways [78]. The cumulative effects of probiotics on reducing inflammation and enhancing tissue regeneration highlight their therapeutic potential in both acute and chronic inflammatory conditions. These findings emphasize the importance of probiotic interventions in managing wounds, preventing infections, and mitigating inflammation-associated diseases [79].

Role of Probiotic in peptic ulcer disease:

Probiotics have been increasingly recognized for their impact in the management of peptiic ulcer disease (PUD) by modulating gut microbiota, reducing *Helicobacter pylori*

(H. pylori) colonization, enhancing mucosal protection, and exerting anti-inflammatory effects Peptic ulcers mainly develop due to Helicobacter pylori infection (H. pylori) with chronic nonsteroidal anti-inflammatory medication (NSAID) usage both of which disrupt the gastric mucosal barrier and lead to ulcer formation. Probiotic strains like *L.bacillus rhamnosus GG* and *Bifidobacteriium bifidum* have demonstrated the capability to inhibit *H. pylori* growth by producing bacteriocins and organic acids that suppress its colonization in the gastric mucosa [80]. Furthermore, probiotics enhance the effectiveness of standard eradication therapy by improving patient compliance and reducing the side effects of antibiotics, such as diarrhea and dysbiosis [81].

Beyond their antimicrobial properties, probiotics contribute to gastric mucosal protection by strengthening the epithelial barrier and increasing mucus production. Certain strains, including *Lactobacillus reuteri* and *Saccharomyces boulardii*, have been shown to enhance gastric mucin expression, which protects the stomach lining from acid and pepsin-induced damage [82]. Additionally, probiotics regulate the secretion of gastric acid and modulate inflammatory pathways, reducing oxiidative strain and infllammation within the gastric mucosa. By downregulating pro-inflammatory markers like TNF-α and interleuken-8 (IL-8), probiotics contribute to mucosal healing and prevent ulcer recurrence [83].

Probiotics also mitigate NSAID-induced gastric damage by enhancing prostaglandin production, which plays impact in maintainiing gastric mucosal in continuity . Studies have demonstrated that probiotic supplementation reduces NSAID-related gastric injury and accelerates mucosal healing by modulating gut microbiota composition and reducing intestinal permeability [84]. Additionally, the gut-brain axis suggests that probiotics may influence stress-related ulcer formation by reducing systemic inflammation and

controlling the hypothalamic-pituitary-adrenal (HPA) axis, participate in streess-induced gastric acid secretion [85]. These findings highlight the multifaceted role of probiotics in PUD management, not only by aiding in *H. pylori* eradication but also by strengthening the gastric mucosa and reducing inflammation. The use of probiotics as an adjunct to conventional therapies holds promise in improving treatment outcomes and reducing ulcer recurrence, emphasizing their therapeutic potential in gastroprotective strategies [86].

Multiple studies have highlighted the protective and healing properties of probiotics in gastrointestinal mucosal damage brought on by aspirin . Research by Altuğ et al. investigated the effects of a probiotic blend on aspirin-induced gastric damage, revealing that its use significantly minimized gastric lesions through various mechanisms. The study reported that probiotics inhibited lipid peroxidation, a key contributor to oxidative stress, which otherwise leads to membrane damage and inflammation, exacerbating mucosal injury. By reducing lipid peroxidation, probiotics help preserve the integrity of the gastric lining. Additionally, the study found that probiotics enhanced mucosal secretory immunoglobulin A (sIgA) production, which plays a critical impact in producing a protectiive barriers against harmful agents, neutralizing toxins, and preventing bacterial adherence to the gastric epithelium. Increased sIgA production reinforces mucosal defense and enhances resistance to injury. Furthermore, the study reported stabilization of mucosal mast cell degranulation, preventing excessive inflammatory responses that could contribute to gastric mucosal damage [87]. Similarly, another study investigated a probiotic mixture containing 13 bacterial strains and observed a significant reduction in gastric damage scores along with enhanced sIgA production, further supporting the role of probiotics in mucosal immunity and ulcer prevention [88]. In another experimental study conducted on Wistar rats, probiotic

treatment improved mucus thickness, re-epithelialization, and gastric gland formation, indicating accelerated tissue regeneration and enhanced ulcer healing [89]. Research on *Lactobacillus rhamnosus* GG demonstrated its role in strengthening the mucosal barrier, promoting mucus secretion, and improving overall mucosal integrity, which facilitated faster recovery from aspirin-induced ulcers [90]. Moreover, probiotics have been shown to regulate inflammatory pathways by downregulating the production of cytokines that promote inflammation, such as IL-6 and TNF-α. This modulation helps reduce gastric inflammation and supports the process of tissue repair [91].Another study highlighted that the administration of *Lactobacillus* and *Bifidobacterium* strains led to an upregulation of gastric prostaglandins, which is having the crucial impact in maintaining mucosal homeostasis and protecting against NSAID-induced damage [92]. Collectively, these findings suggest that probiotics exert pleiotropic effects in mitigating NSAID-related gastric damage through antioxidant, immunomodulatory, and anti-inflammatory mechanisms, making them a promising therapeutic option for preventing aspirin-induced gastric ulcers [93]. Thus, selecting a test drug based on these screening models ensures the identification of the most effective probiotic strains for clinical application, paving the way for novel gastroprotective therapies.

Gastric Ulcer Experimental Models

The pathophysiology of stomach ulcers and possible treatment approaches are investigated using a variety of experimental models. These models fall under the following categories:

1. Ulcer Models Induced by Stress

o Water-Immersion Restraint Ulcer

o Ulcer from Cold-Immobilization Stress

o Ulcer Produced by Restraint

2. Ulcer Models Induced by Drugs

o NSAIDs (Aspirin/Indomethacin)

o Ulcer Caused by Corticosteroid Use

3. Models of Chemically Induced Ulcers

o Chronic Ulcers Induced by Acetic Acid

o Ulcer Induced by Hypertonic Saline

o Alcohol-Related Ulcer

4. Models of Surgically Induced Ulcers

o Yah's Model of Pyloric Ligation-Induced Ulcer

o Duodenal Ulcer Induced by Cysteamine

1. Stress Model-Related Ulcers

A. Ulcer Induced by confinement

• Mechanism: Excessive vagal stimulation brought on by physical confinement causes increased stomach acid output, decreased mucosal blood flow (ischemia), and elevated oxidative stress [(94).]

• Method: This model induces stress-related stomach ulcers in rats by confining them in a tube or tiny cage for two to six hours.

• Uses: This model is used to examine how psychological stress affects the development of ulcers and how the hypothalamic-pituitary-adrenal (HPA) axis contributes to damage to the stomach mucosa.

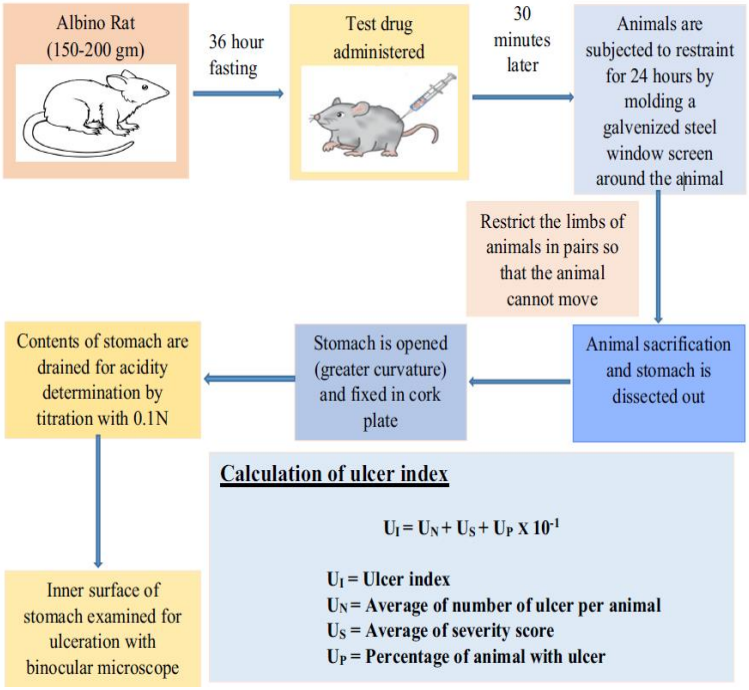


Figure. 3 – Methodology of restrain induced ulcers.[123]

B. **Immobilization by Cold Ulcers from Stress**

• Mechanism: Cold stress causes ischemia, increases acid output, and improves stomach motility [95].

• Method: Rats are kept in this model at 4°C for two to four hours, which causes hypoxia and oxidative stress to cause lesions in the stomach mucosa.

C. Ulcer with Water-Immersion Restraint

• Mechanism: Vagal activation brought on by physical constraint and exposure to cold water causes stomach ischemia and increased acid secretion [96].

• Method: To induce acute stomach erosions, For 4–8 hours, rats are kept in confinement and submerged partially in water at 22–25°C until the xiphoid process occurs.

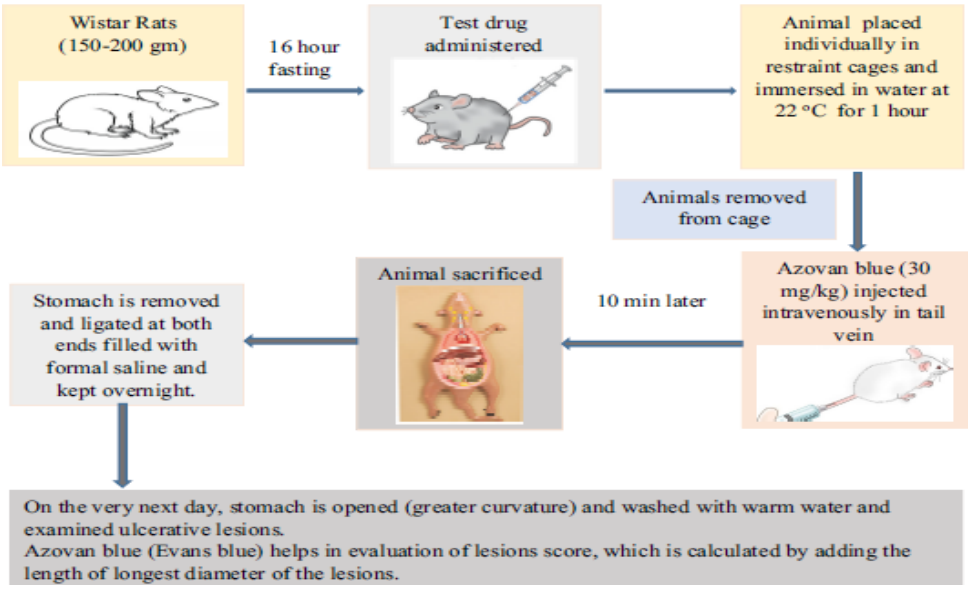


Figure. 4 – Methodology of cold water immersion induced ulcers.[120]

Drug-Induced Models of Ulcer

A. NSAID (aspirin/indomethacin)-induced ulcer

• Mechanism: NSAIDs reduce prostaglandin production via cyclooxygenase-1 (COX-1) inhibition. This decrease leads to tissue damage by weakening mucosal defense, increasing stomach acid output, and elevating oxidative stress [97].

• Method: Oral administration of aspirin either indomethacin (10–30 mg/kg) or 200–400 mg/kg. causes gastric mucosal injury in rats, usually leading to ulcer development within 4–6 hours.

• Uses: This model is frequently used to evaluate the effectiveness of anti-ulcer drugs, encompassing prostaglandin analogs, proton pump inhibitors, and antioxidants having gastroprotective qualities.

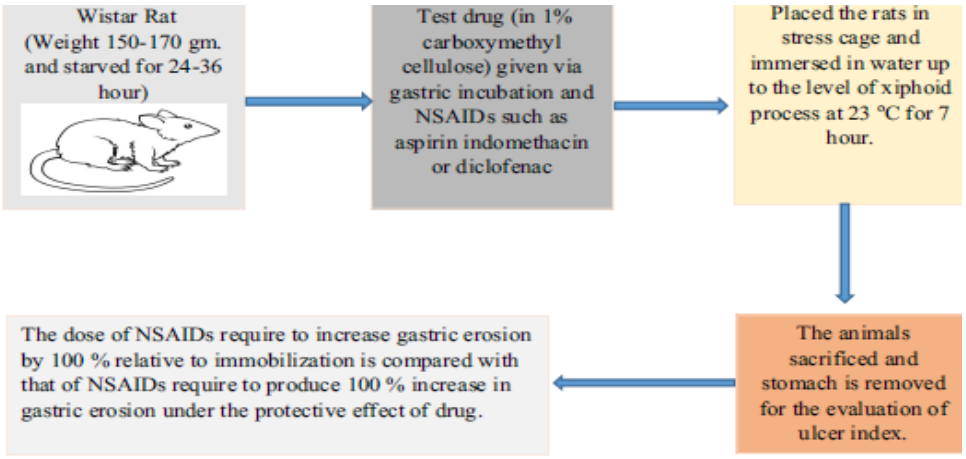


Figure. 5 – Methodology of stress and NSAIDs induced ulcers.[119]

B. The Induction of Ulcer by Corticosteroid

• Mechanism: Prostaglandin activity is inhibited and mucus release is reduced by corticosteroids, which delays ulcer healing [98].

• Method: Either dexamethasone (5–10 mg/kg) or prednisolone (5–20 mg/kg)

causes stomach erosion and slows down the healing process.

3.Models of Chemically Induced Ulcers

A. Mechanism of Ethanol-Induced Ulcer: Ethanol increases vascular permeability, oxidative stress, and inflammation and direct injury to the stomach mucosa [99].

• Method :Oral 100% ethanol (1–5 mL/kg) is administered to rats.

which within an hour causes hemorrhagic gastric lesions.

• Uses: This model is frequently employed to study inflammatory reactions, oxidative stress, and the effectiveness of gastroprotective medications.

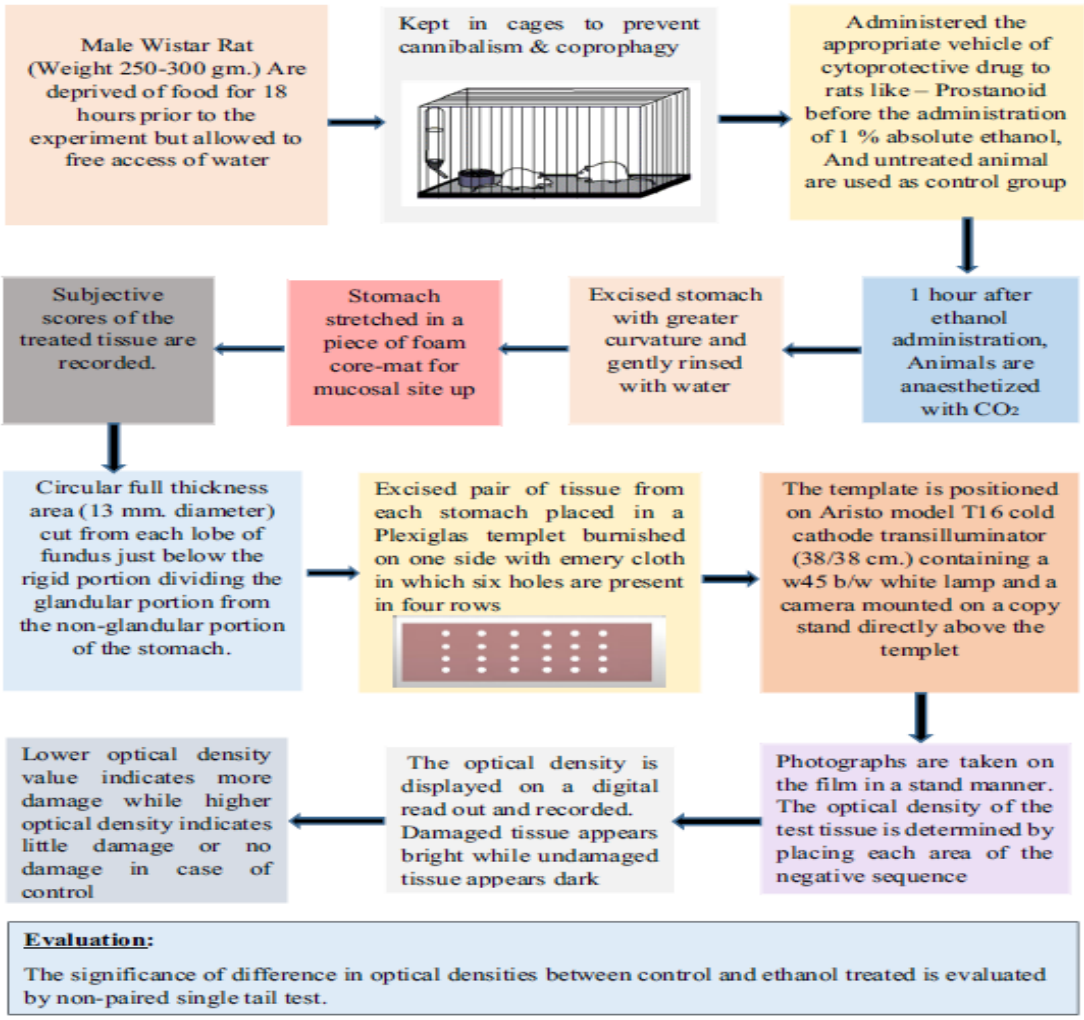


Figure. 6 – Methodology of ethanol induced mucosal damage.[124]

B. Chronic Ulcer Caused by Acetic Acid

• Mechanism: Chronic peptic ulcer disease is strongly resembled by the persistent necrotic ulcers caused by acetic acid [100].

• Method: The stomach submucosa is injected with a tiny Acetic acid content (0.05–0.1 mL, 20–40% concentration), which causes ulcers that last for a few weeks.

• Uses: This model is employed to study angiogenesis, ulcer healing mechanisms, and the long-term impacts of medicinal substances.

C. Ulcer Caused by Hypertonic Saline

• Mechanism: Dehydration and osmotic damage to stomach epithelial cells brought on by hyperosmolarity result in mucosal damage [101].

• Method: Rats are given an oral 5–10% NaCl solution, which causes mucosal lesions to appear quickly.

4. Models of Ulcers Induced by Surgery

A. Ulcer Caused by Pyloric Ligation (Shay's Model)

• Mechanism: When the pylorus is ligated, it prevents the stomach from emptying, which leads to a buildup of acid, an increase in peptic activity, and eventual damage to the mucosa [102].

• Procedure: Under anesthesia, the pylorus is knotted , and the stomach is checked for the development of ulcers and the release of gastric acid 4-6 hours later.

• Uses: This methodology is commonly used to evaluate pH levels, stomach acid secretion, affects the effectiveness of antisecretory medications such H_2-receptor antagonists and proton pump inhibitors (PPIs).

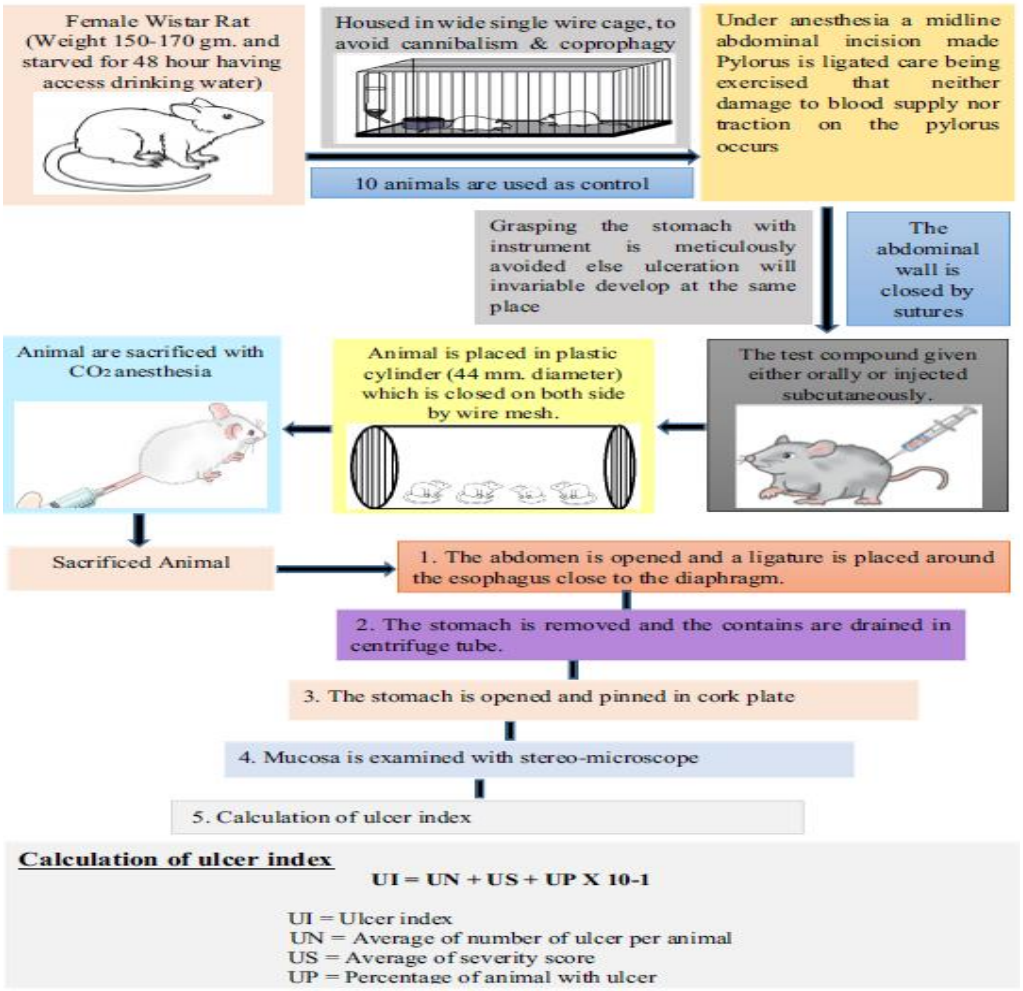


Figure. 7 – Methodology of pylorus-ligated-induced peptic ulcers.[121]

B. Duodenal Ulcer Induced by Cysteamine

• Mechanism: Cysteamine injection causes the duodenum to secrete excessive amounts of acid, which in turn raises oxidative stress and causes inflammation of the mucosa, finally leading to the development of ulcers [103].

• Method: Duodenal ulcers are caused within 24 to 48 hours of oral administration of cysteamine at dosages ranging from 200 to 400 mg/kg.

• Uses: This approach is frequently utilized to evaluate the therapeutic potential of anti-ulcer drugs and investigate the pathogenesis of duodenal ulcers.

Important information on the mechanisms underlying the development of duodenal and stomach ulcers may be gained from each of these experimental models. Through the utilization of chemical, surgical, drug-induced, and stress-induced models, scientists may methodically examine the several elements that contribute to the formation of ulcers. These models are still crucial for the development of pharmacological ulcer prevention and treatment techniques as well as for the advancement of gastroprotective medications.

The NSAID-induced ulcer model serves as an ideal screening tool for evaluating the efficacy of probiotics. By leveraging their antioxidant, anti-inflammatory, and mucosal protective properties, probiotics emerge as a promising therapeutic strategy for preventing aspirin-induced gastric ulcers. This outcome focus impact on probiotics as a viable alternative or adjunct to conventional gastroprotective therapies, paving the way for further clinical investigations into their role in ulcer prophylaxis and management.

METHODOLOGY

This study was carried out following a well-defined experimental protocol. Healthy male adult Wistar rats weighing 150–180 g apiece were purchased from the Animal House of JNMC, Belgaum. Ten days before the trial began, the animals were kept in a controlled laboratory environment with a 12-hour light-dark cycle to facilitate acclimatization. Throughout this period, Food and water were freely available to the rats , ensuring optimal adaptation to the experimental conditions.

Probiotic additives were obtained from **regional pharmacies or reputable online sources**. All **laboratory instruments and reagents** required for the study were procured from **certified suppliers** to ensure quality and reliability.

The study was carried out in complete conformity with the ethical rules established by the Committee for the Control and Supervision of Experiments with Animals (CCSEA). All procedures were designed to ensure the humane treatment, proper care, and ethical handling of the experimental animals, including their sacrifice, in compliance with regulatory standards.

Study Design

This study employs an experimental approach to evaluate the gastroprotective effects of probiotics on male Wistar rats' stomach ulcers caused by aspirin, comparing their effectiveness with the proton pump inhibitor (PPI) pantoprazole, which is frequently administered. All drugs and treatments will be administered orally through an intragastric tube (oral gavage) over a period of 14 days.

Experimental Groups and Treatments

The study is structured into distinct experimental groups to evaluate the gastroprotective effects of probiotics against aspirin-induced gastric mucosal injury while comparing their efficacy with a standard pharmacological treatment.

Group	Treatment	Dosage
I	**Normal Control (Normal Saline)**	**1.8 ml/ kg**
II	**Gastric Ulcer Control**	**1.8ml/ kg**
III	**Standard Treatment: Pantoprazole**	**1.8 mg/kg**
IV	**Probiotic Treatment (Probiotics that have been blended, freeze-dried, or cryopreserved)**	**90 mg/kg or 16.2 × 10^9 CFU (16.2 billion CFU)**

Table 2 – Showing that the experimental group and treatments

Probiotic Strains Administered:

Plantarum, Lactobacillus acidophilus, and Lactobacillus rhamnosus

Casei Lactobacillus

This experimental framework is designed to evaluate the protective potential of probiotics in lowering stomach ulcers brought on by aspirin while determining their relative efficacy compared to a standard proton pump inhibitor treatment.

Induction of Experimental Gastric Ulcers

Twenty-four male Wistar rats were split into four groups at random, with six rats in each group . Each group getting different **pre-treatments over a 14-day period**, as outlined in the study design. To maintain consistency across all experimental conditions Food was

withheld 36 hours prior to administering saline or aspirin, whereas water was provided ad libitum throughout the period. However, to standardize gastric conditions, **water access was withheld for two hours before aspirin administration** (104).

On Day 15, 500 mg/kg of aspirin was administered orally to both the experimental and control groups to cause stomach ulcers, while the normal control group was given normal saline. All substances were administered via intragastric tube oral gavage (105).

This method is effectively **replicated gastric injury caused by aspirin**, providing a controlled platform to evaluate the **gastroprotective potential** of various agents, including **probiotics, pharmacological inhibitors and antioxidants**.

Euthanasia Procedure

By the time the study was over, the rats were humanely euthanized in accordance with CCSEA guidelines. To ensure a painless and ethical procedure, a fatal intraperitoneal dose of Sodium Thiopental (120 mg/kg)—equivalent to Four times the standard anesthetic dose—will be administered. This approach guarantees a swift and humane termination of the animals.

Figure 8 : induction of anaesthesia

Blood Sample Collection

At the conclusion of the study, the rats were humanely euthanized in accordance with CCSEA guidelines. To ensure a humane and controlled procedure, blood will be drawn under anesthesia two hours after gastric ulcer induction.

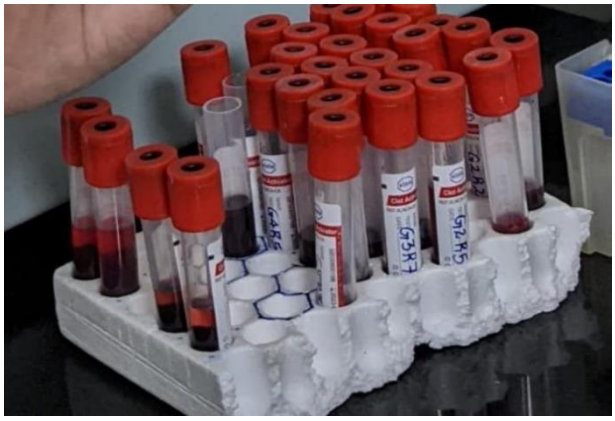


Figure 9 : Collection of blood through vacutainer after cardiac puncture

Figure 10 : Centrifuge process for Plasma separation

Evaluation of inflammatory marker

Five milliliters of blood were drawn in order to measure inflammatory cytokines, including TNF-α, interleukin-2, and interleukin-4, using the ELISA method.

Figure 11 : the dilution of stock solution

Figure 12: ELISA microplate reader machine

RESULTS:

This study aimed to evaluate the effect of probiotics on gastric mucosal lesions caused by aspirin and contrast their efficacy with pantoprazole. Data collected during the study was compiled into an Excel sheet and analyzed Use Version 10.0 of GraphPad Prism. Key parameters, including gastric ulcer index, & inflammatory cytokines were assessed using appropriate statistical tests. A p-value of less than 0.05 was considered statistically significant, with results presented as Mean ± SD. The findings indicate that pantoprazole is significantly more effective than probiotics in reducing inflammatory cytokines and promoting gastric healing. While probiotics demonstrated gastroprotective effects by enhancing mucosal defense mechanisms, pantoprazole showed superior efficacy in ulcer healing by significantly lowering the ulcer index, inhibiting gastric acid secretion, and enhancing mucosal regeneration. These results suggest that while probiotics may provide a complementary protective effect, pantoprazole remains the more effective treatment for aspirin-induced gastric ulcers.

Cytokine	**Normal Control**	**Disease Control**	**Pantoprazole Group**	**Probiotics Group**
TNF-α (pg/mL)	**40.2 ± 2.5**	**175.8 ± 5.3**	**85.6 ± 3.8** # *	**125.4 ± 3.2** *
IL-2 (pg/mL)	**30.4 ± 1.8**	**125.6 ± 4.7**	**65.3 ± 2.9** # *	**95.7 ± 2.6** *
IL-4 (pg/mL)	**65.5 ± 3.2**	**25.2 ± 1.5**	**50.8 ± 2.4** # *	**40.3 ± 3.1** *

Table. 3 - All values are shown as the mean ± standard deviation. Statistical Similarities Tukey's post-hoc test was used after one-way analysis of variance (ANOVA) across groups. Statistical significance was defined as a p-value of less than 0.05.

- A statistically significant difference ($p < 0.05$) from the Disease Control group is shown in the table by ∗, and a statistically significant difference ($p < 0.05$) from the Normal Control group is indicated by #.

Markers of inflammation

At the conclusion of the trial, the levels of interleukin-2, interleukin-4, and serum TNF-α were assessed.

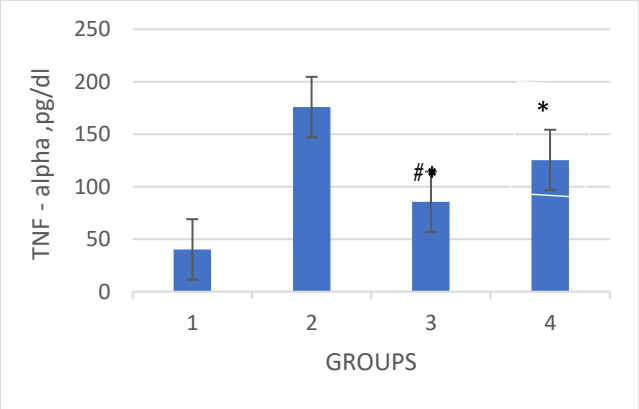
1. TNF-α Analysis

At the end of the study, the TNF-α values for the **Normal Control**, **Disease Control**, **Pantoprazole**, and **Probiotics** groups were **40.2 ± 2.5**, **175.8 ± 5.3**, **85.6 ± 3.8**, and **125.4 ± 3.2 pg/mL**, respectively.

TNF-α levels varied statistically significantly across the groups, according to a one-way ANOVA ($p < 0.001$). Post hoc evaluation using Tukey's test revealed a substantial increase in TNF-α levels in the group under disease control as opposed to the group under normal control ($p < 0.001$). Additionally, TNF-α levels were markedly lower in the Pantoprazole group relative to the group under disease control ($p < 0.001$) . The Probiotics group also showed a significant reduction; however, its effect was less pronounced than that of Pantoprazole ($p < 0.05$). These findings suggest that Pantoprazole exhibited a significantly stronger anti-inflammatory effect by effectively

reducing TNF-α levels, while probiotics resulted in only a slight decrease, highlighting their comparatively lower efficacy.

Graph no 1: The Effect of various drugs on TNF - α levels in Aspirin induced gastric ulcer



Group 1. Normal Control group

Group 2. Disease Control group

Group 3. Pantoprazole-Treated group

Group 4. Probiotics-Treated group

- One-way ANOVA and post hoc Tukey's test were used to examine the results, which are shown as Mean ± SD. A significance level of $p < 0.001$ indicates a notable difference between the Disease Control group and the treatment groups (Pantoprazole and Probiotics).

- A statistically significant difference between the two is indicated by the symbol ∗ Pantoprazole and Probiotics groups, whereas # denotes a significant variation in comparison to the Disease Control group.

2. IL-2 Analysis

At the end of the study, the IL-2 values for the **Normal Control**, **Disease Control**, **Pantoprazole**, and **Probiotics** groups were **30.4 ± 1.8**, **125.6 ± 4.7**, **65.3 ± 2.9**, and **95.7 ± 2.6 pg/mL**, respectively.

A statistically significant difference in IL-2 levels between the groups was shown by a one-way ANOVA ($p < 0.001$). Post hoc Tukey's test revealed a substantial increase in IL-2 levels between the Normal Control group and the Disease Control group ($p < 0.001$) . Moreover, IL-2 levels were much less in the Pantoprazole group compared to the Disease Control group ($p < 0.001$). The Probiotics group also showed a reduction in IL-2 levels; however, the effect was significantly less pronounced than that observed with Pantoprazole ($p < 0.05$).

This conclusion is suggestive that Pantoprazole proved to be markedly more effective in lowering IL-2 levels and regulating immune-mediated inflammation, while probiotics exhibited only a moderate impact, emphasizing their relatively lower anti-inflammatory capacity.

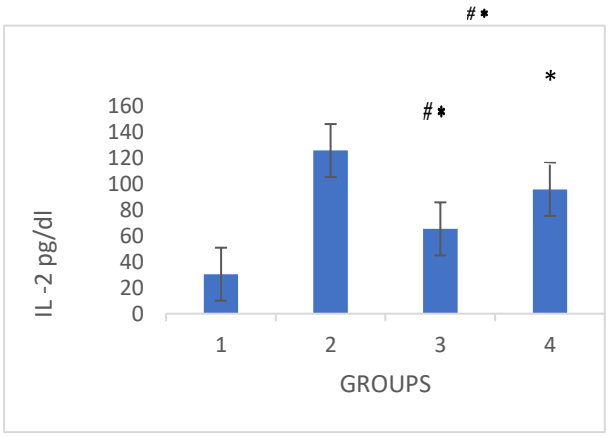
Graph no 2: The Effect of various drugs on IL- 2 levels in Aspirin induced gastric ulcer

Group 1. Normal Control group

Group 2. Disease Control group

Group 3. Pantoprazole-Treated group

Group 4. Probiotics-Treated group



- One-way ANOVA and post hoc Tukey's test were used to examine the results, which are shown as Mean ± SD. A significance level of $p < 0.001$ indicates a notable difference between the Disease Control group and the treatment groups (Pantoprazole and Probiotics).
- A statistically significant difference between the two is indicated by the symbol ∗ Pantoprazole and Probiotics groups, whereas # denotes a significant variation in comparison to the Disease Control group.

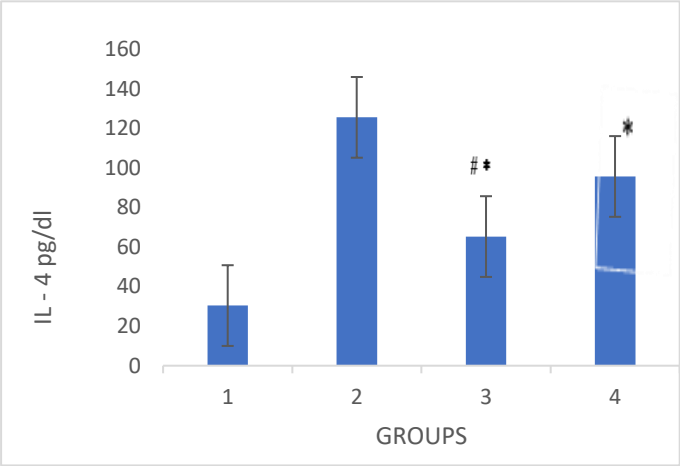
3. **IL-4 Analysis**

At the end of the study, the IL-4 values for the **Normal Control**, **Disease Control**, **Pantoprazole**, and **Probiotics** groups were **65.5 ± 3.2**, **25.2 ± 1.5**, **50.8 ± 2.4**, and **40.3 ± 3.1 pg/mL**, respectively.

A statistically significant difference in IL-4 levels between the groups was shown by a one-way ANOVA ($p < 0.001$). Post hoc Tukey's test indicated a notable decline in IL-4 levels between the Normal Control group and the Disease Control group ($p < 0.001$). Additionally, IL-4 levels were significantly elevated in the Pantoprazole group relative to the Disease Control group ($p < 0.001$). The Probiotics group also showed an increase in IL-4 levels; however, this effect was significantly less pronounced than that observed with Pantoprazole ($p < 0.05$).

These findings suggest that Pantoprazole is more effective than Probiotics in restoring IL-4 levels and facilitating mucosal healing. In comparison, Probiotics showed a limited impact, indicating a weaker role in immune regulation and tissue regeneration.

Graph no - 3: The Effect of various drugs on IL – 4 levels in Aspirin induced gastric ulcer



Group 1. Normal Control

Group 2. Disease Control

Group 3. Pantoprazole-Treated

Group 4. Probiotics-Treated

- One-way ANOVA was used to examine the results, which are shown as Mean ± SD. followed by post hoc Tukey's test. A significance level of $p < 0.001$ indicates a notable difference between the Disease Control group and the treatment groups (Pantoprazole and Probiotics).
- A statistically significant difference between the two is indicated by the symbol ∗ Pantoprazole and Probiotics groups, whereas # denotes a significant variation in comparison to the Disease Control group.

Histopathology Finding:

The stomach was gently opened along the larger curvature to reveal the gastric mucosa at the end of the investigation. The mucosal surface was gently washed with cold PBS to eliminate any residual blood before further examination. Ulcer formation was then assessed, and the Ganguly AK technique was used to determine the ulcer index :[122]

Ulcer Index = $(UP \times 10^{-1}) + UN + US$

In the case: UI = Ulcer Index

UN stands for average ulcers per animal.

US stands for "mean severity score."

UP stands for the percentage of animals that have ulcers.

- The following categories were used to classify ulcer severity:
- Normal mucosa that shows no signs of harm (0.0)
- Mild reddish discolouration (0.5)
- Spot lesions are seen in 1.0 cases;
- Hemorrhagic streaks are in 1.5 cases;
- Ulcers measuring 3 mm² to < 5 mm² are in 2.0 cases; and

Group	UN (Ulcerated Rats)	US (Mean Ulcer Score)	UP (Total Ulcers)	Ulcer Index
Normal Control	0	0.000	0	0.000
Gastric Ulcer Control	6	4.583	24	18.332
Pantoprazole Treated	3	1.667	7	3.890
Probiotics-Treated	5	1.583	9	2.849

- Ulcers larger than 5 mm² are in 3.0 cases.

Table 4 – Ulcer Index

Pantoprazole, a Gastric acid is efficiently suppressed by proton pump inhibitors (PPI).

secretion, which is a key factor in ulcer formation. In contrast, probiotics mainly work through **mucosal protection and modulation of inflammation**, which, while beneficial, do not provide the same level of acid suppression. This explains why Pantoprazole demonstrated a superior therapeutic effect in preventing aspirin-induced gastric damage.

Gross Examination:

Group 1 - Normal group

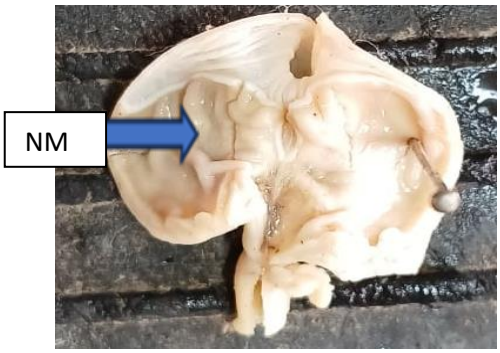


Figure 13. - Gross appearance of stomach with normal mucosa (NM)

Group - 2 Disease Control group

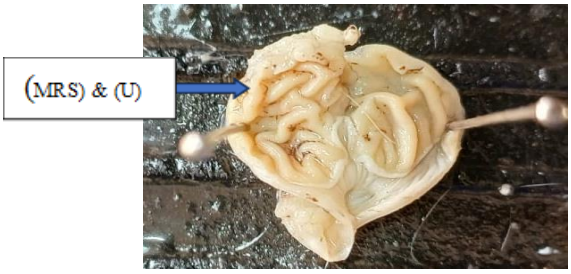


Figure 14. – Gross images shows that stomach along with mucosa showing multiple red spots (MRS) and ulcers (U)

Group 3 - Pantoprazole treated group

Figure 15 - Gross image of stomach shows that mucosa showing few red spots(FRS)

Group 4– Probiotic treated group

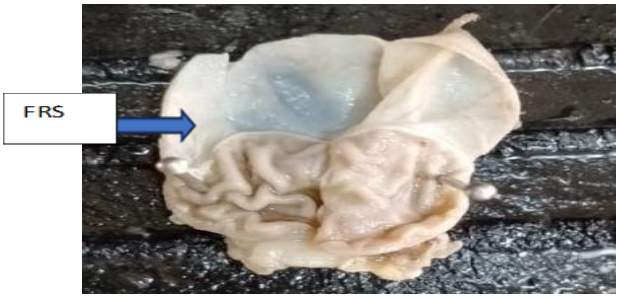


Figure 16 - Gross image of stomach shows that mucosa showing few red spots

Microscopic Examination

Group1: Normal Control group

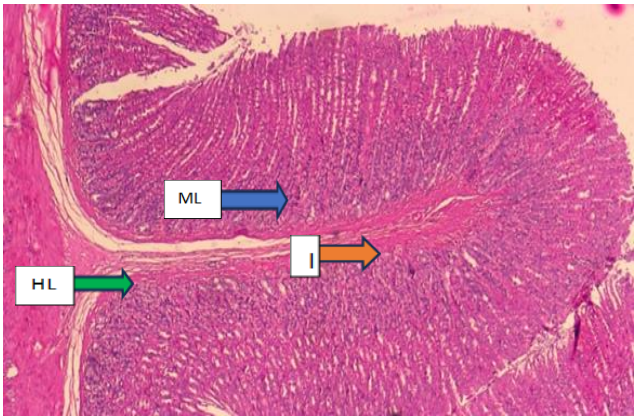


Figure. 17 - Histological analysis of the stomach mucosa in the normal control group (H&E stain, x200) revealed a well-preserved mucosal layer (ML) with no visible signs of damage or inflammation (I). The epithelial lining remained intact, with normal glandular architecture and no evidence of ulceration or haemorrhagic lesions (HL).

Group 2 – Disease Control Group:

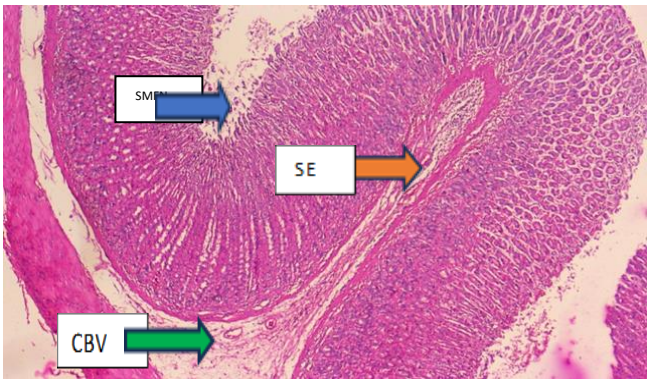


Figure. 18 - Histological analysis of the stomach mucosa in the gastric ulcer control group revealed notable pathological changes. The findings included (1) disruption of the superficial mucosal layer with focal necrosis (SMFN), (2) submucosal edema (SE) , and (3) congestion of blood vessels (CBV) accompanied by a moderate mixed inflammatory cell infiltrate. These observations were made using Hematoxyliin and Eosin (H and E) staining at x200 magnification.

Group 3 – Pantoprazole-Treated Group

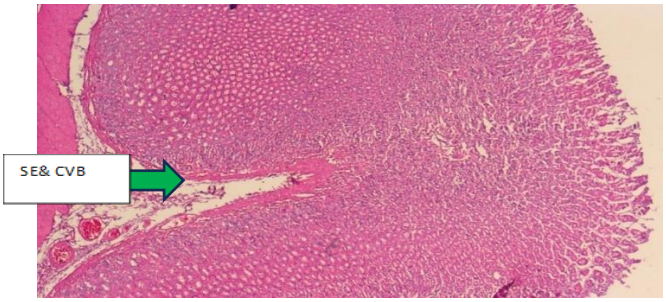


Figure. 19 - Histological analysis of the gastric mucosa of the pantoprazole-treated groups demonstrated the presence of submucosal oedema (SE) and congested blood vessels (CBV). These findings were observed using Haematoxylin and Eosin (H&E) staining at x200 magnification.

Group 4 – Probiotic treated group

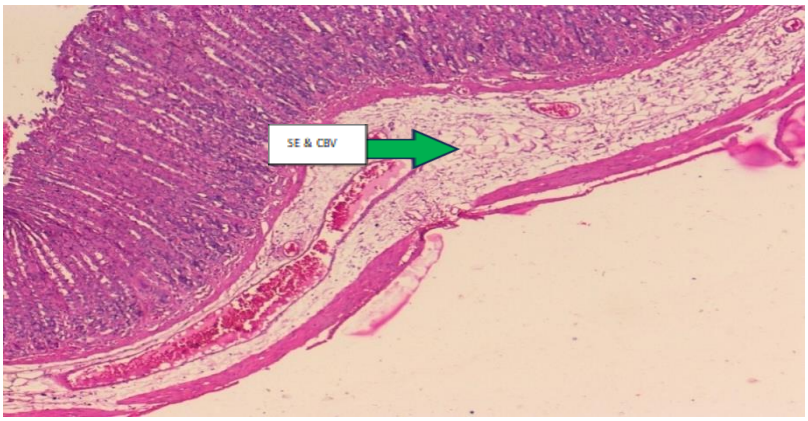


Figure. 20 - Histological analysis of the gastric mucosa of rats in the probiotic-treated group exhibited submucosal oedema (SE) and congested blood vessels (CBV).

DISCUSSION

Peptic ulcer disease (PUD) is a prevalent digestive condition marked by damage to the stomach or duodenum's mucosa as a result of a disparity between defensive and offensive forces. Numerous factors contribute to the pathophysiology of PUD, such include increased stomach acid production, prolonged use of nonsteroidal anti-inflammatory medications (NSAIDs), Helicobacter pylori infection, and compromised mucosal defense systems.. Due to their extensive usage for pain relief and inflammation control, NSAID-induced stomach ulcers continue to pose a serious therapeutic concern. Chronic usage of NSAIDs, especially aspirin, has been shown to induce ulcers and damage to the stomach mucosa.

The suppression of cyclooxygenase-1 (COX-1), a crucial enzyme involved in prostaglandin production, is the primary process via which NSAIDs cause gastrointestinal damage. By promoting increasing the release of mucus and bicarbonate, enhancing mucosal blood flow, and inhibiting the generation of stomach acid, prostaglandins are essential for preserving the stomach mucosa's integrity. When these defense mechanisms are inhibited, mucosal sensitivity to acidic and proteolytic damage is heightened, which can result in ulcer development [(106).]

A common nonsteroidal anti-inflammatory medicine (NSAID), aspirin is well recognized for its antipyretic, antiplatelet, and analgesic effects. Despite its widespread therapeutic applications, prolonged aspirin use is associated with significant gastrointestinal complications mostly ulcer development and damage to the stomach mucosa. Inhibition of cyclooxygenase-1 (COX-1), an enzyme essential for the production of protective prostaglandins (PGs), is the main mechanism causing this harm.

These prostaglandins are essential for maintaining the stomach mucosa's integrity by encouraging the production of mucus and bicarbonate and making sure that the mucosal blood circulation is sufficient. When COX-1 is inhibited, the production of these protective substances decreases, leading to impaired mucosal defense. Consequently, the gastric environment becomes more hostile, with reduced mucus protection, diminished bicarbonate buffering capacity, and increased gastric acid secretion. This renders the gastric mucosa highly vulnerable to acid-mediated injury and proteolytic damage caused by pepsin, ultimately resulting in mucosal erosion and ulceration [106].

In addition to impairing mucosal defense, aspirin further exacerbates gastric injury by promoting increased production of reactive oxygen species (ROS) as a result of oxidative stress. These very active chemicals set off a series of processes that cause proteins, lipids, and nucleic acids to sustain oxidative damage. A strong inflammatory response is brought on by the resulting oxidative imbalance, which is manifested by immune cell activation, pro-inflammatory cytokine production, and neutrophil infiltration into the stomach mucosa.

[107]**García-Rayado** & Sostres highlighted that this inflammatory reaction is a key contributor to mucosal rupture , haemorrhagic damage, and the prolonged repair of gastric ulcers. The persistent inflammatory process worsens the ulcerative injury and hinders the natural regenerative mechanisms of the gastric epithelium.

NSAID-induced It is possible to efficiently heal stomach ulcers. pharmacologically with proton pump inhibitors (PPIs), such as pantoprazole. These medications work by permanently blocking The parietal cells of the stomach contain the H^+/K^+ ATPase enzyme.

Consequently, less gastric acid is produced. The environment is more conducive to

mucosal healing when stomach acidity is suppressed. Long-term PPI usage is linked to a number of possible disadvantages, notwithstanding its effectiveness. [108] **Mal et al. emphasized that** chronic PPI therapy can lead to alterations in gut microbiota composition, impaired nutrient absorption (notably Ca, Mg and vit B12), and increased risk of enteric infections due to hypochlorhydria.

Due to concerns surrounding prolonged PPI use, researchers have investigated alternative and adjunctive therapies, including probiotics, as a means to enhance gastroprotection and mitigate the adverse effects of PPIs. [109]**According to Lien et al.,** probiotics can help restore microbial balance within the gastrointestinal tract, reinforce mucosal defense mechanisms, and regulate immune responses, making them a promising approach for preventing NSAID-induced gastric ulcers. Probiotics, composed of beneficial live microorganisms, contribute significantly to fostering a balanced microbiome to improve gut health and preventing the overgrowth of pathogenic bacteria. [110] **Byun et al. reported** that probiotics could effectively reduce NSAID-induced small intestinal damage by modulating inflammatory pathways and stimulating mucus secretion, thereby fortifying mucosal protection against ulcer formation. This study goal is to assess Probiotics' potential for gastroprotection in contrast to pantoprazole using gastric ulcer model can caused in male Wistar rats by aspirin. The protective effects of interventions were analyzed based on pro - levels of inflammatory cytokines (IL-2, IL-4, TNF-α) and histological changes in the stomach epithelium . [111] **Antonenko & Beregova demonstrated** that while probiotics significantly alleviated gastric mucosal injury, they were not as effective as pantoprazole in reducing inflammatory markers, particularly TNF-alpha and IL - 2 .

Inflammatory cytokines acts pivotal effect in the pathogenesis and progression of gastric injurys. [112] **Iijima highlighted** that TNF - α and interleukin - 2 contribute to ulcer severity by promoting leukocyte infiltration, oxidative stress, and apoptotic damage to gastric epithelial cells, whereas IL-4 serves as an anti-inflammatory mediator that aids in tissue repair and mucosal healing .

TNF-α, a major pro-inflammatory cytokine, is particularly important in ulcer formation due to its role in exacerbating gastric inflammation, oxidative stress, and epithelial apoptosis**.** [113] **According to Kotob et al.,** rats given aspirin showed noticeably higher TNF-α levels, which were associated with tissue damage and severe mucosal inflammation. Treatment with pantoprazole dramatically decreased levels of inflammatory cytokines (IL-2, IL-4, TNF-α) and histological changes in the stomach epithelium , although their impact was not as strong or quick as pantoprazole's, indicating a more gradual control of the inflammatory response.

Histopathological evaluations provided deeper insights into the severity of gastric injury and the protective effects of different treatment interventions. [114] Kron noted that rats given aspirin showed significant pathological damage, such as glandular atrophy, inflammatory cell infiltration, necrosis, epithelial erosion, and hemorrhagic damage.. The loss of gastric epithelial integrity left the mucosa highly susceptible to further acid-induced injury, exacerbating ulcer formation .

Pantoprazole was shown to be more efficient than probiotics in reducing aspirin-induced stomach damage in a comparative study of treatment effectiveness. [115] **Pilotto et al. discovered** that rats given pantoprazole showed notable improvements in histopathology, a considerable increase in IL-4 expression, and a significant drop in TNF-α and IL-2 levels**..** These findings support the notion that pantoprazole's ability to

directly suppress gastric acid secretion facilitates a more immediate and robust mucosal healing response. Conversely, probiotics exerted a better gradual prophylactic effect, basically via gut microbiota regulation, immune modification , and reinforcement of mucosal defenses rather than direct acid suppression . [116] **According to Sayed et al.,** probiotics were helpful in lowering inflammation and maintaining mucosal integrity, although their protective benefits were not as noticeable or quick as those of pantoprazole.

The combined results of these studies suggest that although probiotics have important gastroprotective advantages, they might not be enough to cure NSAID-induced ulcers on their own. [117] **García-Rayado** & Sostres noted that probiotics primarily contribute to long-term gastric health maintenance rather than the acute healing of ulcerative lesions . In addition to higher Pro-inflammatory mediator levels (TNF-α, IL-2) and decreased IL-4 production the aspirin-treated group showed substantial gastric mucosal injury, which was shown by extensive erosions, necrosis, inflammatory infiltration, and glandular rupture.

[108]**Mal et al. confirmed** that pantoprazole treatment led to significant histopathological improvements, reductions in inflammatory markers, and restoration of mucus production, reaffirming its role as a frontline pharmacological option for NSAID-induced ulcers.

Probiotics also demonstrated gastroprotective effects by modulating inflammation, promoting mucus release, and protect the mucosal surface. [109] **Lien et al. proposed** that an optimized treatment strategy could involve the combined use of pantoprazole and probiotics— where probiotics support stability of the microbiota and long-term stomach protection, while pantoprazole offers instant acid control.Probiotic strains with improved

gastroprotective qualities should be the main focus of future research, along with possible synergistic effects between PPIs and probiotics, as well as clinical studies to validate these findings in human subjects. [115] **Pilotto et al.** suggested that incorporating probiotics as an adjuvant management for gastric ulcers caused by NSAID presents a promising approach for achieving shielded and more exclusive ulcer treatment.

LIMITATIONS OF THE STUDY:

While this study come up with significant insights into probiotic-mediated gastroprotection, many limitations exist. The use of Wistar rats may not fully imitate in human ulcer pathology, and the short study duration does not show chronic NSAID use. Only a particular probiotic blend was evaluated, and optimal dosage was not explored. Mechanistic insights were limited, as inflammatory cytokine level were evaluated but not fully analyzed at the molecular level. Histopathological evaluation could have been more explained, and long-term ulcer recurrence was not evaluated. The study also has deficiency of comparison with possible gastroprotective agents and did not appraise gut microbiota Dispersion. Lastly, human clinical research are needed to authenticate these findings.

Future studies should survey long-term probiotic impact, optimal dosages, additional probiotic strains, molecular mechanisms, and conduct human clinical trials to authorize probiotics as a corresponding therapy for stomach ulcers brought on by NSAIDs.

CONCLUSION :

This research indicates that the main consequences of aspirin-induced stomach ulcers are oxidative stress, inflammation, and mucosal damage. Pantoprazole successfully lessened the severity of ulcers . via reducing inflammation and acid production, demonstrating its effectiveness as a treatment. However, because long-term PPI use causes issues, alternative strategies are required. Despite being less effective than pantoprazole, probiotics (L. acidophilus, L. plantarum, L. rhamnosum, and L. casei) have demonstrated considerable gastroprotective benefits, lowering inflammation and bolstering mucosal defense. Probiotics show potential as a supportive therapy for long-term stomach protection, even if they might not be able to completely replace PPIs in acute instances.

Future studies have to concentrate on strain-specific probiotics and clinical trials in order to maximize their efficacy in the prevention and treatment of ulcers brought on by NSAIDs.

Probiotics and PPIs combined could provide a safer and more thorough ulcer therapy approach.

SUMMARY :

Peptic ulcers (PUD) are a prevalent stomach condition caused by the protective and aggressive forces in the mucosa functioning in different ways. One of the primary elements that contribute to the creation of stomach ulcers is NSAIDs, particularly aspirin.

impairment of the mucosa Aspirin reduces the formation of defensive prostaglandins (PGE_2) via cyclooxygenase-1 (COX-1) inhibition. This leads to a decrease in mucus and bicarbonate secretion, an increase in stomach a rise in oxidative stress and the formation of acid.

The stomach mucosa is more susceptible to harm as a result of these modifications , which can result in inflammation, ulcers, and lesions on the stomach mucosa. PPIs like Pantop, which promote ulcer healing and significantly lower HCl secretion, are the conventional therapy for ulcers caused by NSAIDs. Long-term PPI usage, however, has been linked to a number of negative consequences, including dysbiosis of the gut microbiota, nutritional malabsorption, rebound acid hypersecretion, and an elevated risk of infections . As a result, alternative or complementary therapies have become more and more popular, For example, probiotics are living microbes. with anti-inflammatory, anti-oxidative, and gastroprotective qualities.

This experimental investigation's goal was to look at the effectiveness of probiotics and pantoprazole in treating intestinal lesions caused by aspirin in male Wistar rats. Four groups participated in the study (I) normal control; (II) control of gastric lesions caused by aspirin; (III)group treated with pantoprazole; and (IV) group treated with probiotics. The probiotic group received 90 mg/kg of a mixture of Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus rhamnosum, and Lactobacillus casei for two weeks, whereas the pantoprazole group received 1.8 mg/kg.

The findings showed that aspirin treatment significantly damaged the stomach mucosa, demonstrated by hemorrhagic lesions, necrosis and infiltration of inflammatory cells, and epithelial erosion. An active inflammatory response was also suggested by the fact that aspirin administration increased inflammatory mediators including Tumor Necrosis Factor-alpha (TNF-α) and Interleukin-2 (IL-2) while lowering Interleukin-4 (IL-4) . Pantoprazole treatment demonstrated substantial anti-inflammatory and mucosal-protective characteristics by considerably reducing TNF-α and IL-2 levels, elevating IL-4 expression, and significantly reducing stomach mucosal damage. By improving the integrity of the stomach mucosa and lowering inflammatory indicators, probiotic administration also demonstrated significant gastroprotective benefits.

On the other hand, It was less effective than pantoprazole. Although the preventive impact of probiotics was more gradual, probably regulated by immunological modulation, microbiota control, and improved mucus secretion, pantoprazole promoted fast ulcer healing predominantly by directly suppressing stomach acid output. These findings imply that probiotics by themselves might not be a sufficient treatment for stomach ulcers brought on by NSAIDs.

However, by enhancing mucosal defenses, lowering inflammation, and maybe minimizing the detrimental long-term consequences of prolonged PPI use , they might be used in conjunction with PPIs as a supplemental strategy. In order to validate their role in the treatment

of NSAID-induced gastric ulcers , future research should concentrate on finding conducting clinical studies in human populations, looking at possible synergistic interactions between probiotics and PPIs, and using probiotic strains with higher gastroprotective potential

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KAHER, BELAGAVI
(Formerly known as K.L.E. UNIVERSITY)
JAWAHARLAL NEHRU MEDICAL COLLEGE,
NEHRU NAGAR, BELGAUM-590010, (KARNATAKA).
INSTITUTIONAL ANIMAL ETHICS COMMITTEE.
Phone No. JNMC (0831)-2471350

Dr.(Mrs)P.P.Patil
Chairperson, IAEC.
Prof & Head Physiology,
J.N.Medical College, Belagavi

Dr. Manish Barvaliya
Main Nominee - CPCSEA
Scientist –E ,ICMR,NITM,
Belagavi

Dr.(Mrs)Netravathi A Kavi
Member - Secretary IAEC
Asso Prof of Pharmacology
J.N.Medical College, Belagavi

CPCSEA Reg.No.: 627/PO/Re/S/02/CPCSEA

MEMBERS:

Dr.Shabbir Rafik Pendhari
Dept.of Pharmacology,
BV Medical College& Hospital
Sangli.

Mr.Atul R Chopade
Dept.of Pharmacology
Rajarambapu College of
Pharmacy, Kasegaon, Sangli

Dr.(Mrs)M.A Vagarali,
Officer Incharge,
Central Animal House,
JNMC, Belagavi.

Dr.V.S.Shirol,
Prof of Anatomy.
JNMC, Belagavi

Dr.M.C.Singanalli
Veterinarian, JNMC,
Belagavi

Dr.Prabhakar Adake
Link Nominee CPCSEA.
Dept of Pharmacology &
Toxicology
KAHER, JGMM Medical College,
Hubli

CERTIFICATE

This is to certify that the project proposal no **19/1** entitled **"Effect of Probiotics on Aspirin induced Gastric Mucosal Lesion (Gastric Ulcer) in comparison to Pantoprazole in Male Wistar Rats : An experimental study"** submitted by [redacted], Pharmacology under guidance [redacted], Pharmacology, JNMC has been approved/recommended by the IAEC of JNMC, Belagavi in its meeting held on **15/04/2023** and **24 male Wistar rats** have been sanctioned under this proposal for a duration of next **12 months**.

Authorized by	Name	Signature	Date
Chairman	Dr Parvati Patil	Patil	15.4.23
Member Secretary	Dr. Netravathi A Kavi		15/4/2023
Main Nominee of CCSEA	Dr. Manish Barvaliya		15/04/2023

No.25/1/99 – AWD (Pt.)
Government of India
Ministry of Statistics & Programme Implementation
(Committee for the Purpose of Control and Supervision of Experiments on Animals)
*****

Shastri Bhavan, New Delhi-110001.
Dated the 19th June 2002.

To -

The Prinicipal/Director/Dean
K.L.E. Society's Jawaharlal Nehru Medical College
Nehru Nagar
Belgaum - 590 010
Karnataka

Subject: **Registration of Establishments/ Breeders under Rule 5(a) of the "Breeding of and Experiments on Animals (Control and Supervision) Rules 1998".**

Sir/Madam,

With reference to your application on the above-mentioned subject, this is to inform that your Establishment is hereby registered for "Research". Your Registration Number is **627/02/a/CPCSEA**. The nominee of CPCSEA on the Institutional Animal Ethics Committee (IAEC) of your Establishment will be intimated in due course.

1. You are requested to quote the above Registration Number in all your future correspondence with the Committee.
2. You are also requested to convene IAEC meeting at the earliest.
3. For further correspondence you are requested to contact Office of CPCSEA at Chennai, at the address given below:

Office of the CPCSEA,
Ministry of Statistics & Programme Implementation
3rd Seaward Road, Valmiki Nagar,
Thiruvanmiyur, Chennai-600 041 (Tamil Nadu)

Yours faithfully,

(R.K.JAIN)
MEMBER SECRETARY (CPCSEA) / DIRECTOR (AW)
Tel. No.3381498

Copy to: - Ms. Prema Veeraraghavan, Expert Consultant (CPCSEA), 3rd Seaward Road, Valmiki Nagar, Thiruvanmiyur, Chennai

3) Dr. Shabbir Rafik Pendhari Department of Pharmacology Bharati Vidyapeeth (Deemed to be University) Medical College and Hospital, Sangli. 416414 Contact No :9766417420 Email :itsshabbir@gmail.com	Scientist from outside the Institute
4) Mr. Atul Ramchandra Chopade Dept of Pharmacology, Rajarambapu College of Pharmacy, Kasegaon, Tal: Walwa, Dist. Sangli – 415404, Maharashtra Contact No :9226346106 Email :chopadearv@gmail.com	Socially Aware Nominee

(Please note that any change in IAEC members can be made only with prior approval of CPCSEA.)

5. The IAEC is valid for a period of five years and is coterminous with renewed period of registration. IAEC is required to be reconstituted at the time of renewal of registration as per CPCSEA guidelines.

6. You are requested to convene the meeting of the re-constituted IAEC within a period of 30 days and upload the same on the website of the CPCSEA.

7. It is stated that only above approved IAEC members shall sign, with date, on the attendance sheet of the IAEC meetings, and decisions will be taken only in meetings where quorum is complete. The quorum for holding IAEC meeting is six (6), and Main Nominee, Scientist from outside the Institute and Socially Aware Nominee must be present in such meetings. Link Nominee can attend in case main nominee conveys his unavailability in writing to the chairman IAEC. However, the Link Nominee should be invited once a year to update him/ her about the activities of the IAEC. Any decision taken in the meetings of IAEC without quorum shall be considered invalid.

8. It is also to inform you that before commencing any research on large animals you are required to send research protocols with due recommendation of IAEC to CPCSEA for further approval (procedure for submission of Research Protocols is available on the website of CPCSEA).

Yours Sincerely,

(Dr. S. K. Dutta)
Member Secretary (CPCSEA)

Copy for necessary action to: Nominees of CPCSEA.

The Main Nominee is requested to ensure that the IAEC meetings are held regularly as stipulated in the SOP of CPCSEA and submit the Annual Inspection Reports of the Animal House Facility regularly on the Website of CPCSEA.

No. 25/373/2010-AWD
Government of India
Ministry of Fisheries, Animal Husbandry and Dairying
Department of Animal Husbandry and Dairying
O/o Committee for the purpose of Control and Supervision of Experiments on Animals
(CPCSEA)
*****

Delhi Milk Scheme Complex,
Shadipur, Delhi – 110008
Date: 19.12.2022

To,

Dr Parwati Patil, Chairperson, IAEC
K.L.E.Society's Jawaharlal Nehru Medical College Nehru Nagar,
Belgaum - 590 010 Karnataka
Email: docparwati@yahoo.co.in
Mobile: 9449019436

Subject: Renewal of Registration and Reconstitution of Institutional Animals Ethics Committee (IAEC)-regarding

Madam,

The registration of Animal House Facility of your establishment with CPCSEA has been **renewed for a period of five years from the date of issue of this letter**.

2. The registration number of Animal House Facility of your establishment is **627/PO/Re/S/02/CPCSEA for Research for Education purpose on small animals.** Henceforth, the registration number may kindly be quoted in all your future correspondence.

3. The CPCSEA has accepted the following members recommended by the establishment.

Name of the IAEC Members	Designation in IAEC
1) Dr.Parwati .P.Patil	Biological Scientist, Chairperson
2) Dr.Netravathi A Kavi	Scientist from different biological discipline, Member Secretary
3) Dr.Veereshkumar S Shirol	Scientist from different biological discipline
4) Dr.Mohan C Singanalli	Veterinarian
5) Dr.Manjula A Vagarali	Scientist Incharge of Animal House Facility

4. CPCSEA hereby nominates the following members to the Institutional Animals Ethics Committee (IAEC) of your establishment:

Details of Nominee(s)	Nominated as
1) Dr. Manish Barvaliya Scientist-E, ICMR-National Institute of Traditional Medicine (NITM) Nehru Nagar, National Highway No. 4 Belagavi590010, Karnataka Contact No :9726901845 Email :drmanishbarvaliya@gmail.com	Main Nominee
2) Dr. Prabhakar Adake Professor of Pharmacology, KAHER's JGMM Medical College, Kotgonadhunshi,Gabbur cross, Hubballi-580028 Karnataka Contact No :9886554800	Link Nominee