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Comparative study of the possible gastroprotective effect of probiotic (*lactobacillus plantarum)* and ranitidine on indomethacin induced gastric ulcer in adult male albino rat

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Abstract

Background and Objective: Indomethacin is an anti-inflammatory drug which causes peptic ulcers as a result of its complication, For this reason, multiple experimental studies have been performed to search for new agents in order to treat or prevent gastric and duodenal ulcers. Probiotics are live microorganisms, and it has been stated by different studies that these bacteria have antioxidant and anti-inflammatory effects so; the aim of the present study is to compare the gastroprotective potential of probiotic *lactobacillus plantarum*, versus the anti-ulcer drug (ranitidine), against indomethacin-induced gastric ulcer in rats.

Materials and methods: Thirty-two adult male rats were divided equally into four groups. Group I: (normal control) animals received distilled water. Group II animals received indomethacin to induce ulcer. Group III: animals were given ranitidine followed by indomethacin. Group IV: animals were also treated with probiotic *lactobacillus plantarum*. Animals of all groups received the treatments orally. Rats were sacrificed 7hr later, and their blood and stomachs were isolated for examinations.

Results: our study revealed that *Lactobacillus plantarum* has ulcer healing properties possibly via enhancement of gastric defense system through increasing PGE2 levels. Further to this, *Lactobacillus plantarum* reduced serum TNF-α, serum IL-Iβ, serum IL-6 and tissue TBARS expression and thus exhibited antioxidant and anti-inflammatory effects and consequently reduced neutrophil infiltration and thus free radicals' generation as ranitidine treated group (p<0.05).

Conclusion: This study demonstrates that compared to ranitidine, Lactobacillus *plantarum* showed gastroprotective efficacy against indomethacin-induced peptic ulcer through possing anti-inflammatory, antioxidant and anti-apoptotic properties

Keywords: **Gastric ulcer, Indomethacin, Ranitidine, probiotic, lactobacillus plantarum.**

Introduction

Peptic ulcers are a digestive tract pathology that includes both duodenal and gastric ulcers. Lesions known as ulcers are lesions that penetrate muscularis mucosa to their full depth. The factors that contribute to morbidity and mortality were one of its main drawbacks (**Amandeep et al., 2012**).

When the "aggressive" and "protective" forces at the luminal surface epithelium are out of balance, peptic ulcers can form. Helicobacter pylori, which is one of the most common causes of peptic ulcer disease and is thought to be present in half of the world's population, hydrochloric acid (HCl), pepsins, NSAIDs, bile acids, alcohol, smoking, and hypoxia are among the aggressive factors. Conversely, protective factors, which have a major global impact on a wide range of people (**Siddique et al., 2018**).

Non steroid anti-inflammatory drugs are typically given for the treatment of a variety of illnesses such Rheumatoid arthritis and osteoarthritis because of their analgesic, anti-inflammatory, and antipyretic effects. Gastric ulcers may occur as a side effect in 35–60% of patients, and their action is mediated by the suppression of prostaglandins, cyclo-oxygenases (cox), and leukotriene production (**Gambero et al., 2005**).

The introduction of selective cyclo-oxygenase (COX) blockers has not been able to eliminate the serious problem of ulcers linked to NSAIDs. Most NSAIDs cause stomach discomfort, mucosal erosion, and ulceration to variable degrees, but relative stomachic toxicity is the main factor to take into account (**Fong et al., 2015**).

Peptic ulceration is typically treated medically by either inhibiting HCL acid secretion or by neutralizing the acid. Numerous pharmacological agent categories have been thoroughly tested for their efficacy in treating acid peptic diseases (**Waller et al., 2005**).

The most often given medications for stomachic lesions brought on by NSAIDs are H2-receptor antagonists. Because they belong to a class of pharmaceuticals that prevent histamine from acting on the parietal or oxyntic cells in the stomach's histamine H2 receptors, H2-receptor

antagonists, such as nizatidine and ranitidine, are antihistamines. Stomach acid production is reduced as a result. They are used to treat gastric reflux disease and peptic ulcer disease (**Panula et al., 2015**).

Probiotics may be utilized to cure stomach ulcers, according to numerous research. Gram-negative bacteria colonization happened quickly at the location of the ulcer in a rat model of acetic acid-induced stomach ulcer, which greatly slowed ulcer healing. However, gram-positive bacterial colonization aided in ulcer healing. Notably, administering the exogenous probiotic strain Lactobacillus sped up the healing of ulcers (**Khoder et al., 2016**).

Eating live bacteria (Lactobacillus bulgaricus) in yoghurt or fermented milk enhances the biological characteristics of the gastrointestinal system. Probiotics are live microorganisms that, when given to a host in sufficient quantities, have a positive impact on their health, according to the Food and Agriculture Organization and the International Scientific Association for Probiotics and Prebiotics (**Hill et al., 2014**).

Previous studies looked at the probiotics' preventative and therapeutic benefits on a number of gastrointestinal and non-gastrointestinal disorders. Probiotics have proven potential therapeutic effects in various metabolic illnesses, such as hyperlipidemia or hypercholesterolemia, obesity, and diabetes, in addition to their traditional advantages for gastrointestinal functions. Probiotic use may therefore help to lower the risk of atherosclerosis and hypertension (**Huang et al., 2014**).

Several research conducted over the past few decades have suggested that probiotics may have a part in the treatment and prevention of cancer. According to data, persons with colon cancer have distinct changes in the composition of their gut's microbes (called dysbiosis). When 1,2-dimethylhydrazine is used to induce colon cancer in rats, there is a large dysbiosis that may be prevented by taking Lactobacillus salivarius Ren orally, effectively suppressing the development of colon cancer (**Zhang et al., 2015**).

The probiotics Bacillus subtilis and Clostridium butyricum have been shown to prevent the growth of 1,2-dimethylhydrazine-induced colorectal cancer in mice. Little is known regarding the potential link between probiotics and carcinogenesis in terms of stomach cancer. Probiotics have been shown to have highly promising anti-proliferative and pro-apoptotic effects on gastric cancer cells, according to certain in vitro studies. Additionally, research has shown that probiotics may help cancer patients avoid the negative side effects of chemotherapy and radiation treatment (**Haghshenas et al., 2014**).

The potential use of probiotics as adjuncts or even replacements for oral antibiotic therapy has been raised, particularly considering the rising incidence of antibiotic resistance. Antibiotics can cause resistance when used repeatedly and without a prescription, and they can also damage the

gut bacteria. In these circumstances, administering probiotics may help the patient recover by restoring the natural microflora, displacing the pathogenic resistant bacteria, and competing with them (**Crouzet et al., 2015**).

Some genetically modified probiotic strains with skills for the release of anti-inflammatory cytokines, vaccines, and anti-pathogenic compounds have been created using novel methods. Probiotics have the potential to be used in the future as delivery systems for gastrointestinal mucosal lesions. 'Pharmabiotics' refers to this innovative method of probiotic-based targeted medicine delivery (**Cano et al., 2014**).

Probiotics may be helpful for gastrointestinal colic, acute infectious diarrhoea, inflammatory bowel syndrome, diarrhoea brought on by antibiotics, travellers' diarrhoea, lactose malabsorption, and inflammatory bowel illnesses, according to some studies. Data on the potential link between probiotic supplementation and gastric ulcer healing and prevention, however, are scarce (**Marchesi et al., 2016**).

The present work aims to evaluate the gastroprotective effect of antiulcer drugs (ranitidine as H2-receptor antagonists) versus probiotics *lactobacillus plantarum* and their ability to treat of NSAIDs-induced peptic ulcer in adult male albino rat.

Materials and Methods

Animals and ethical decision

Thirty two male albino rats of local strains weighing between (120 - 140 g) and aged 6 to 8 weeks. They were kept for two weeks in a conventional laboratory setting with a temperature of 25 °C, relative humidity of 60 %, and an equal day and night cycle, as well as unrestricted access to a standard pellet diet and unlimited access to water. The care and usage of laboratory animals were handled in compliance with international standards during the study's approach.

Drugs and experimental design

Drugs and chemicals

Both probiotic lactobacillus plantarum and ranitidine were bought from Sigma Pharmaceutical Company in Quesna. All other reagents and kits were of the analytical quality and were acquired from Sigma-Aldrich Co. in St. Louis, Missouri, USA.

Experimental design

Rats were assigned into 4 equal groups (n=8/group). Regular weight checks were performed.

Group 1: Control group which received physiological saline 1m for 10 days

Group 2: Indomethacin group which received physiological saline for 10 days then on day 11 was received single dose of indomethacin (50mg/kg,i.p)

Group 3: Probiotic + indomethacin group Which received lactobacillus plantarum 1ml/kg for 10 days then on day 11 received single dose of indomethacin (50m/kg,i.p)

Group 4: Ranitidine + Indomethacin group which received ranitidine (5mg/kg) for 10 days then received on day 11 indomethacin single dose (50m/kg,ip), Sample gathering and animal sacrifice.

Rats were slaughtered using isoflurane inhalation anesthesia. Blood samples were collected by heart puncture into vacuum tubes, and serum was separated by centrifuging blood at 4,000 rpm for 20 min. Until further biochemical investigation, serum was stored at -80°C in plastic Eppendorf containers. The stomach was removed, weighed, and cleaned in a phosphate buffer solution. The right one was immediately kept at -80 °C for subsequent measurement of PGE2 and TBARS.

Biochemical investigations

With the help of the diamond diagnostic kit (**Diamond Diagnostics Company, Egypt**), Plasma tumor necrosis factor-alpha (TNF-α), IL-6 and IL-1β were measured by ELISA kit (**Life Technologies Corp., Frederick, MD, USA**).

Enzyme-linked immunosorbent assay (ELISA)

After collecting the stomach tissues from the animals, cold phosphate buffer saline (**Sigma Aldrich, USA**) was used for keeping the gastric tissue samples that were then homogenized and centrifuged using a cool ultra-centrifuge. Later, the supernatants were collected and utilized in the ELISA study to measure the levels of PGE2 (**Cat. No. E0504Ra, Bioassay Technology Laboratory, Shanghai, China**) and TBARS (**Cat. No. E1369Ra, Bioassay Technology Laboratory, Shanghai, China**).

Statistical analysis

Utilizing (Graph Pad Prism version 9.4 for Windows, 2010, Graph Pad software, Inc.), the results' statistical analysis was carried out. ATN score was reported as median, and data were presented as mean + standard error of the mean (SEM). One-way ANOVA was used to compare several groups, followed by the post hoc Tukey test, the Games Howell test, and the Bartlett test for groups with uneven variances. The threshold for significance was set at the 0.05 level of probability.

Results

Plasma TNF- alpha, IL-6 and IL-1β

As shown in Table 1; the probiotic-treated group showed insignificant changes in the serum TNF- alpha (Figure 1), serum IL-Iβ (Figure 2), and serum IL-6 (Figure 3) when compared with their corresponding ranitidine treated-group values. The probiotic and ranitidine-treated groups recorded a significant elevation (P-value<0.05) in the TNF- alpha, IL-Iβ, and IL-6 in the serum as compared with the control group while showing improvement when compared to indomethacin induced group.

Tissue PGE2 levels

As shown in Table 1 and Figure 4; the probiotic and ranitidine-treated groups exhibited significant elevation in PGE2 level (P-value <0.05) when compared with the indomethacin group. On the other hand, indomethacin caused a significant reduction in PGE2 level in comparison with the control group. Moreover, PGE2 levels of both ranitidine and probiotic groups were comparable to each other without a significant difference.

Tissue TBARS level

As shown in Table 1 and Figure 5; oxidative stress is known to contribute to the pathogenesis of gastric ulcers and hence the tissue levels of TBARS were measured in this study as markers of oxidative stress and found to be significantly elevated (*P*-value<0.05) in the indomethacin group when compared with the control group. On the other hand, TBARS levels in the probiotic and Ranitidine groups were significantly reduced in comparison with the indomethacin group.

Table (1): The plasma concentration of tumor necrosis factor (TNF-α), interleukin (IL-6), IL-1β, tissue PGE2 and tissue TBARS in control and treated rats

All data are expressed as mean ± SEM. Different letters are significantly different at p≤0.05.

Parameters	**Group 1** Control	**Group 2** Induced	**Group 3** Probiotic treated group	**Group 4** Ranitidine treated group
TNF (pg/ml)	35.2±0.90 ^{c}	90.2±9.9^{a}	50.2±0.5^{b}	55.1±0.8^{b}
IL-6 (pg/ml)	75.4±3.7 ^{c}	160.9±9.60^{a}	110.5±4.20^{b}	105.9±4.2^{b}
IL-1β (pg/ml)	0.71±0.01^{c}	1.31±0.02^{a}	0.9±0.05^{b}	0.98±0.02^{b}
PGE2	5.5±0.01^{a}	1±0.01^{c}	4.3±0.25^{b}	4.1±0.14^{b}
TBARS (mmol/g tissue)	0.65±0.001^{c}	16.9±0.04^{a}	5.7±0.03^{b}	5.56±0.1^{b}

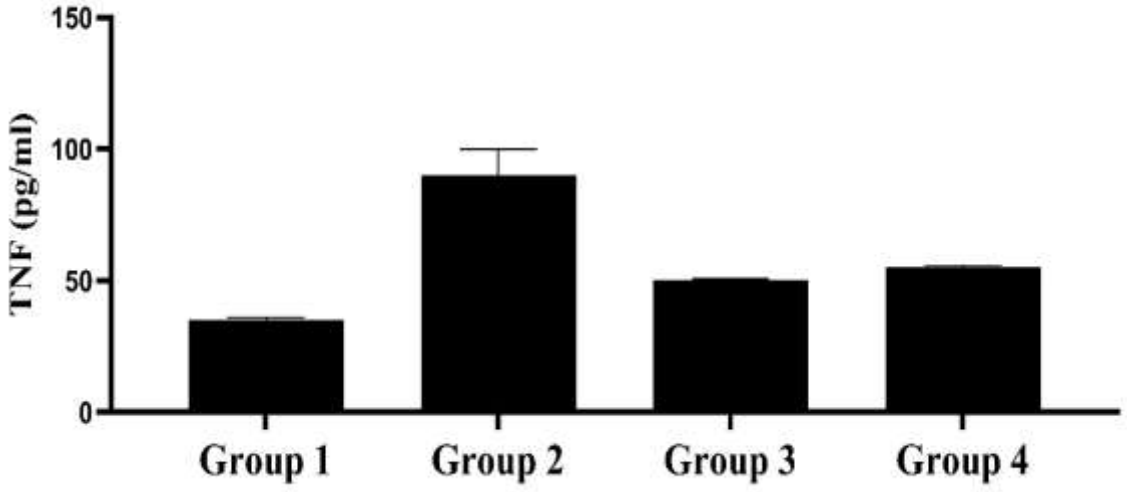

Figure (1): TNF (pg/ml) in control and treated rats

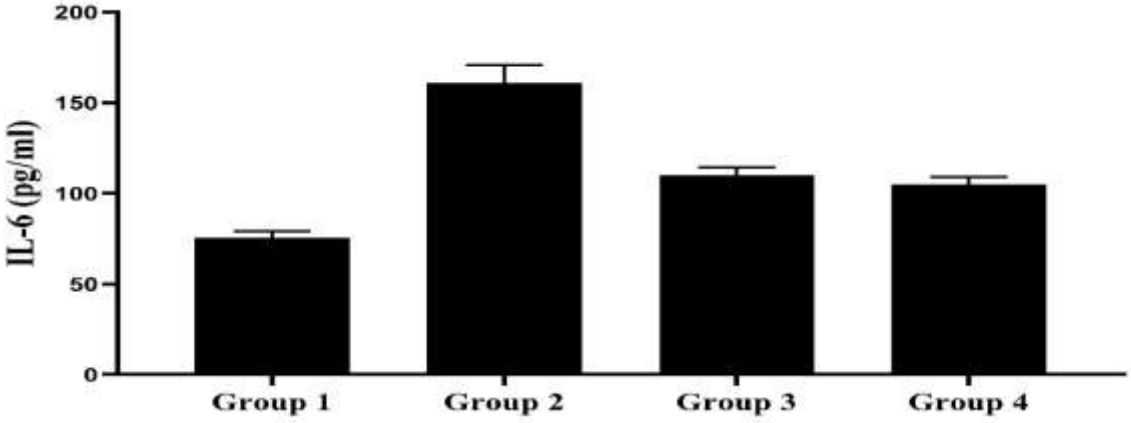

Figure (2): IL-6 (pg/ml) in control and treated rats

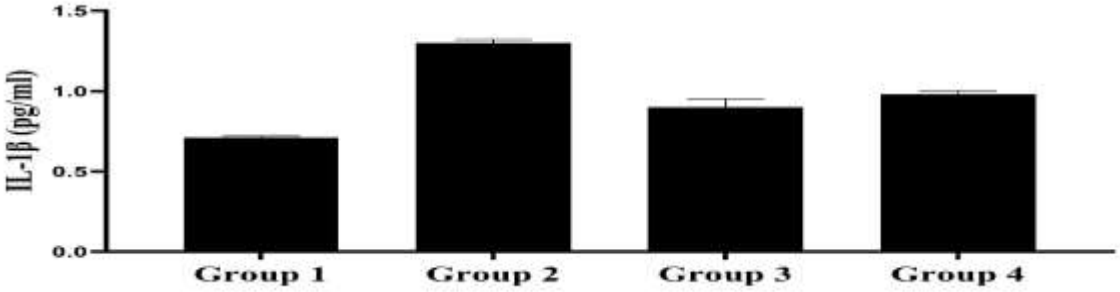

Figure (3): IL-1β (pg/ml) in control and treated rats

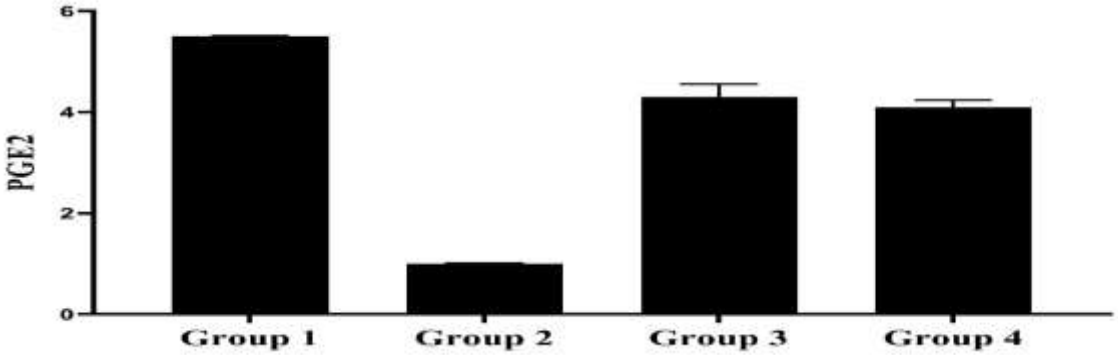

Figure (4): Tissue PGE2 levels in control and treated rats

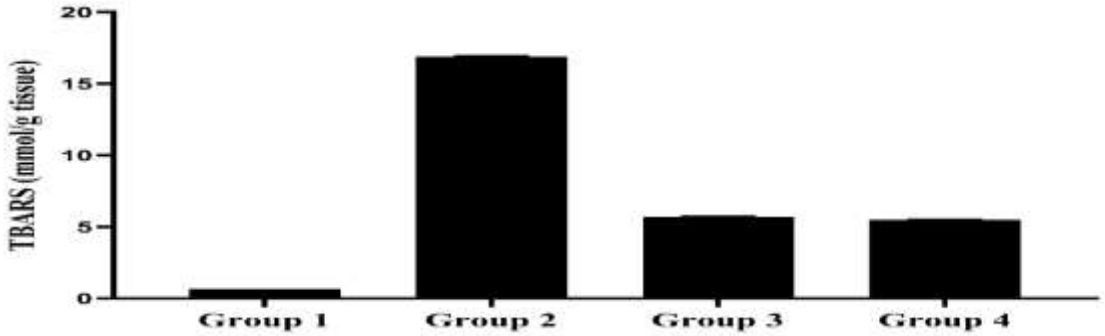

Figure (5): Tissue TBARS level in control and treated rats

Histological and histochemical studies

Stomachs were taken, fixed in neutral buffered formol saline and they were processed by paraffin technique, to be subjected to histological and histochemical examination.

Five slides from each specimen with 2 levels on each slide were cut at 5-µm thicknesses. Haematoxylin and Eosin (H&E) stain: For general morphological and structural study.

A minimum of 10 fields for each slide were examined and for severity of changes they scored semiquantitatively the scoring was done as none, mild, moderate and severe changes. **(Drury and Wallington 1980)**

Periodic acid Schiff (PAS) and Alcian blue stains to show the mucous barrier layer of the stomach mucosa for measuring its thickness and optical density **(Pearse, 1977).**

2. Results

2.1. **Histological Results**

Group I (control): Microscopic examination of H&E stained sections showed normal appearance In G1 the mucosa was healthy. There were no ulcers no hemorrhage and no blood congestion. Both the upper surface of the mucosa and lower part of the mucosa the glands boundaries are intact. The cells of the glands have vesicular nuclei. The lamina propria was rich in eosinophils . (Fig.1 A).

Group II (Indomethacin group): The results obtained by H&E staining showed marked blood vessels dilatation and congestion. There was marked loss of the normal shape of the glands. The cells showed vaculation and nuclear pyknosis. There was disappearance of eosinophils from the lamina propria. (Fig. 1B)

Group III (Probiotic and Indomethacin group): the glands boundaries were intact. It contained healthy cells with vesicular nuclei but there were scattered cells with pyknotic nuclei. There were also some dilated blood capillaries and some lymphocytic infiltration. It is noted that in the base of the mucosa there were disappearance of eosinophils from lamina propria (Figure 1C).

Group IV (Rantidine and Indomethacin group): the glands start to retain their shape but there were some cells in the glands still showing vaculation and nuclear pyknosis. There was also disappearance of eosinophils from the lamina propria. (Figure 1D).

PAS & Alcian blue stain results

In the stomach body In G1 (figure 2A): the surface of the mucosa was covered with well-formed PAS positive layer without interruptions. In G2 (figure 2B): There were more remnants of PAS positive layer that cover surface of the mucosa. In G3 (figure 2C): the surface of the mucous was covered with well-formed PAS positive layer except for few interrupted area. In G4: There were remnants of PAS positive layer that cover the surface of the mucosa (Figure 2D)

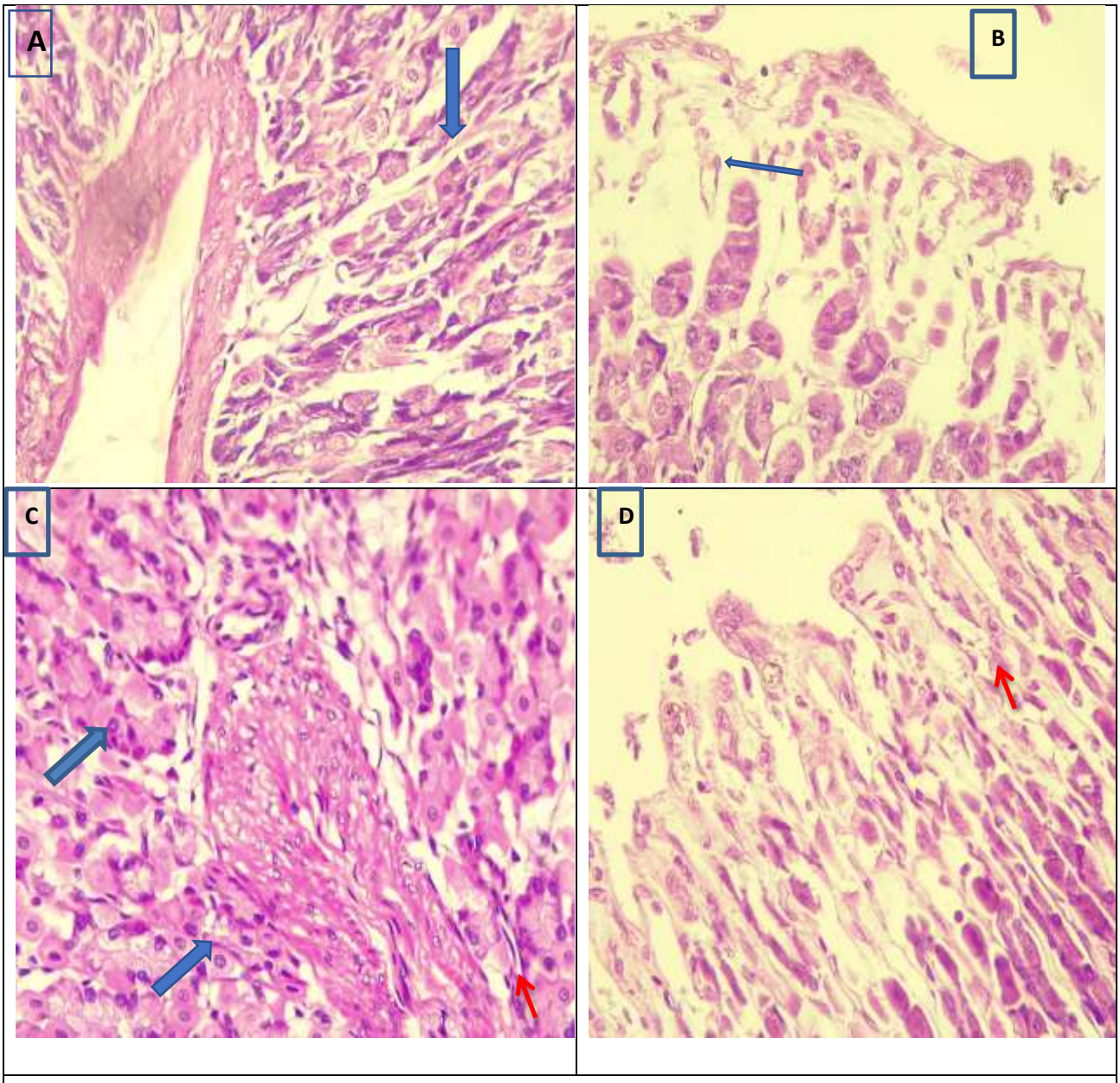

Figure 1: Photomicrographs of a section in the mucosa of stomach body of rats, the mucosa is intact and intact cells containing vesicular nuclei (blue arrows) in G1 (A).Wide spread necrotic cells with pyknotic nuclei (blue arrows) in G2 (B). The mucosa appear intact and healthy cells with vesicular nuclei (red arrows) and few necrotic cells with pyknotic nuclei (blue arrows) in G3 (C). Many necrotic cells with pyknotic nuclei (blue arrows) in G4 (D). (x400 H & E)

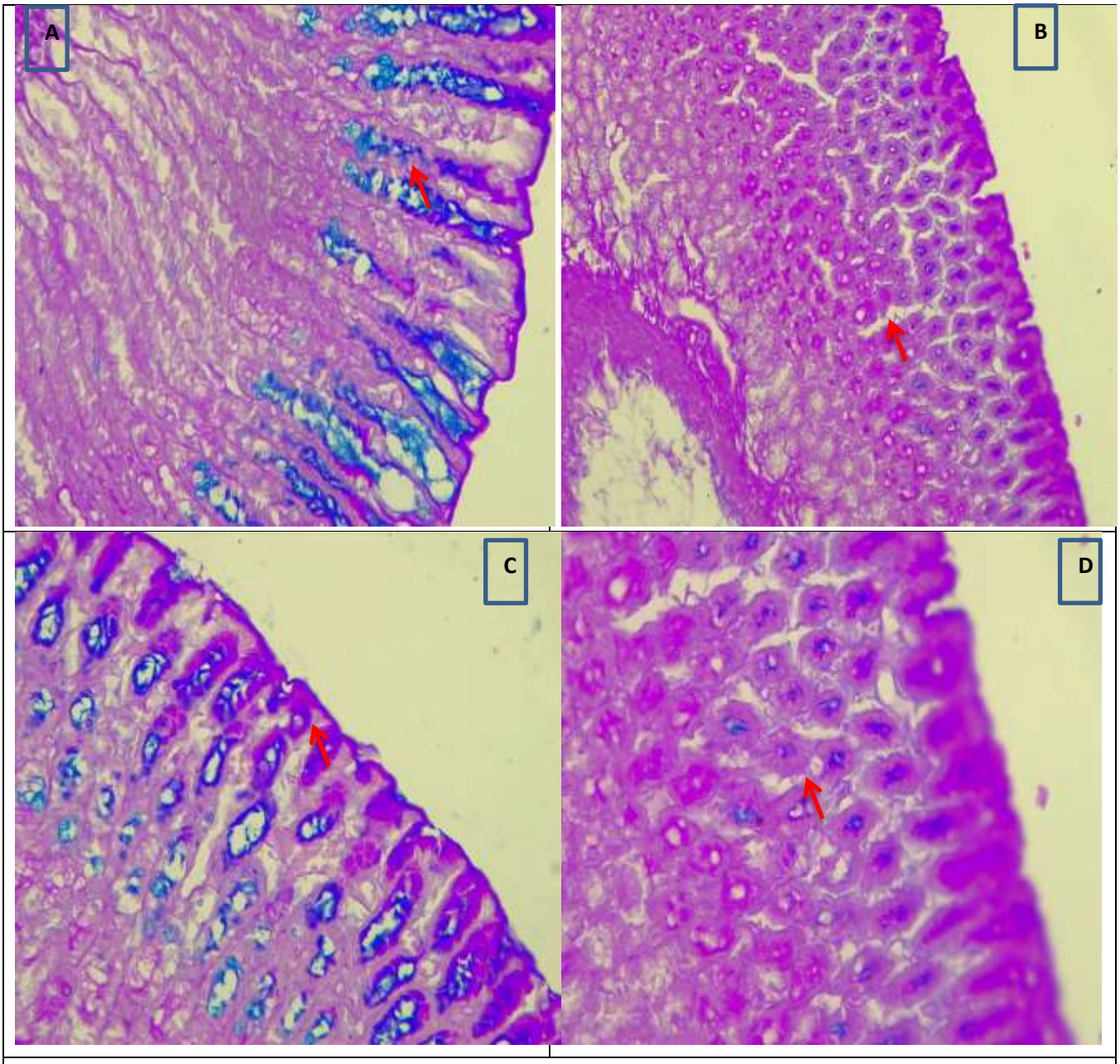

Fig 2 : Photomicrographs of sections in stomach body of rats showing PAS positive mucous barrier (red arrows) in G1 (A) . Remnants of PAS positive mucous barrier (red arrows) in G2 (B). The remnants are increased in G3 (C). Well-formed PAS positive mucous layer (yellow arrows) with interrupted areas in G4 (D). x400 PAS & Alcian Blue

Discussion

Gastric ulcers, often referred to as peptic ulcers, form within the stomach's epithelial lining because of the mucus layer being thinned out by increased acid and pepsin release. Helicobacter pylori infection, alcohol misuse, smoking, and long-term use of non-steroidal anti-inflammatory medicines (NSAIDs) are some of the main causes of these erosion processes in the stomach (**Toljamo et al., 2011**).

South Korea has one of the highest yearly incidences of perforated peptic ulcers in the 21st century, according to **Azhari et al.'s (2018)** systematic study on the global incidence of peptic ulcers. Proton pump inhibitors or the complete eradication of H. pylori, which involves antibiotic therapy, are still the only clinical options available for treatment. The majorities of the prescribed medications, like proton pump inhibitors, reduces stomach acid production and disrupt the protective pH barrier, which increases the risk of enteric infections brought on by bacterial pathogens like Clostridium difficile, Salmonella spp., and Campylobacter spp. As a result, additional complementary treatments are required to treat stomach ulcers while simultaneously shielding the gastrointestinal tract against enteric infections (**Imhann et al., 2017**).

Probiotics are defined as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" and include various strains of the lactic acid bacteria (LAB), including several Lactobacillus spp. Numerous studies have been done on the effectiveness of different Lactobacillus strains in treating stomach ulcers (**Khoder et al., 2016**) either as single therapeutic agents or in combination with antibiotics (**Goderska et al., 2018**).

Through the up regulation of prostaglandin E2, the augmentation of mucus secretion, or the modulation of inflammatory responses, most of the examined strains have been found to inhibit or protect the gastric mucosal barrier (**Şenol et al., 2014**). Additionally, some of these probiotics not only have preventive benefits but also therapeutic ones by promoting angiogenesis, enhancing epithelial development, and up-regulating the expression of anti-inflammatory cytokines. Currently, strategies involving either "new" antimicrobials or probiotics are seen as promising strategies for treating or preventing stomach ulcers (**Goderska et al., 2018**).

According to several researches, probiotics' regulating influence on the gut microbiota may be one of the potential underlying mechanisms for their effect on the host's physiology (**Ji et al., 2018**). However, a more thorough understanding of the effect of probiotic therapy on treating stomach ulcers is still inadequate and requires more research and improvement. One of the three main short chain fatty acids (SCFAs) produced by the intestinal microbiota, butyrate, is thought to protect against the development of stomach ulcers brought on by ethanol, according to current research. Butyrate pretreatment reduced the levels of the pro-inflammatory cytokines IL1, TNF, and IL6 and increased the production of stomach wall mucus (**Liu et al., 2016**).

In the current investigation, indomethacin was employed to induce a gastric ulcer and both histological abnormalities in the gastric mucosa and gross morphological injuries to the stomach were the results. The protective benefits that numerous experimental drugs displayed against indomethacin-induced ulcers have been determined to be due to both raising stomach mucosal PGE2 levels and decreasing free radical production (**AlKreathy et al., 2016**).

In comparison to the control group, the Indomethacin group had significantly lower PGE2 levels. This impact is ascribed to non-selective inhibition of the COX enzyme, which is thought to be the underlying mechanism for the previously described indomethacin-induced stomach ulcers. Additionally, ulcers brought on by indomethacin were avoided when misoprostol, a PGE2 analogue, was exogenously supplied. According to reports, PGE2 increases mucin synthesis, enhances blood flow, and reduces stomach acid output to improve gastric protection (**Suleyman et al., 2010**).

As opposed to the indomethacin-treated group, treatment of ranitidine led to a considerable rise in PGE2 levels. This result is consistent with a prior study that showed ranitidine greatly raised PGE2 levels in the stomach tissues, increased mucin synthesis, and eventually decreased the size of ulcers (**El-Saka et al., 2014**).

L. plantarum prevents TNF- from causing epithelial barrier disruption and IL-8 release. Probiotics may maintain the function of the epithelial barrier and reduce inflammation by changing the signal transduction pathway (**Ko et al; 2007**).

When compared to the indomethacin control group and showing insignificant results with their corresponding Ranitidine treated-group values (P-value 0.05), the probiotic group showed a significant increase in PGE2 concentration and a significant decrease in serum TNF-, serum IL-I, serum IL-6, and tissue TBARS. This is in agreement with **Wu et al**'s studies from 2021, which showed that the protective effect of Lactobacillus plantarum The biochemical indicator detection revealed that LP-HFY09 boosted levels of somatostatin (SS), prostaglandin E2 (PGE2), glutathione (GSH), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and glutathione (GSH), while decreasing levels of malondialdehyde (MDA).

Additionally, LP-HFY09 increased levels of the anti-inflammatory cytokine IL-10 while decreasing levels of the pro-inflammatory cytokines interleukin (IL)-1, IL-6, and tumour necrosis factor (TNF). The quantitative polymerase chain reaction (q-PCR) examination revealed that LP-HFY09 enhanced the mRNA expression of nuclear factor E2-related factor 2 (Nrf2) and downstream genes, including copper/zinc superoxide dismutase (SOD1), heme oxygenase-1 (HO-1), gamma-glutamylcysteine synthetase (GSH1), manganese superoxide dismutase (SOD2), catalase (CAT), and GSH-Px. According to this study, LP-HFY09 effectively reduced inflammation by boosting the gastric mucosa defence factor, decreasing oxidative stress, and suppressing the inflammatory response in alcohol-induced stomach ulcers (**Wu et al; 2021**).

Our findings supported the findings of **Aboutalebi et al. (2010)**, who showed that Lactobacillus plantarum significantly reduced stomach ulcer area relative to control and negative control groups and improved ulcer healing. On day 5 after the production of the ulcer, a histological analysis revealed a much lower number of neutrophils and a significantly higher number of macrophages and fibroblasts ($p<0.001$). On day 14 following ulcer induction, a substantial decrease in neutrophil, macrophage, and fibroblast numbers was also seen ($p<0.001$).

To ascertain the changes in oxidative stress and lipid peroxidation brought on by ulcers, the levels of TBARS in the gastric tissue were evaluated by ELISA. When compared to the control group, the indomethacin group's TBARS levels were shown to be higher. This result is consistent with a previously published study in which the treatment of indomethacin caused the formation of ulcers as well as an increase in TBARS levels, which were explained by the oxidation of phospholipid (**Aal-Aaboda et al; 2010**).

On the other hand, ranitidine therapy decreased the level of TBARS in the stomach tissue by lowering lipid peroxidation through its anti-secretory and antioxidant activities, which mitigated tissue damage. Isrogladin maleate has a protective effect against ulcer-induced tissue injury, and Jaldani and colleagues demonstrated that this effect was synergistic when paired with ranitidine (**Jaldani et al; 2017**).

This may help to explain why using Lactobacillus plantarum had a protective effect against stomach ulcer induction. In addition, another study compared the effects of Lactobacillus plantarum and ranitidine in the prevention of gastrointestinal ulceration. This study found that *Lactobacillus plantarum* considerably reduced oxidative stress as seen by the reduction in TBARS levels.

IL-1 expression was examined to better understand the inflammatory changes associated with stomach ulcers, and it was discovered that this expression was considerably higher in the indomethacin group than in the control group. Alternately, ranitidine administration decreased IL-1 expression because of ranitidine's ability to heal ulcers, which is consistent with earlier findings (**Ugan and Un; 2020**).

Gastric mucosal IL-1 levels were observed to be considerably lowered in response to Lactobacillus plantarum. Because LT receptor antagonists decrease the expression of IL-1 and other inflammatory cytokines that are generated by tumor necrosis factor-alpha (TNF-), this effect may be explained (**Ferreira et al; 2016**).

Conclusion

According to the study's findings, *Lactobacillus plantarum* may have the ability to cure ulcers by boosting the stomach defense system by raising PGE2 levels. Additionally, Lactobacillus plantarum showed antioxidant and anti-inflammatory activities, which in turn reduced neutrophil infiltration and the subsequent production of free radicals. It also decreased the expression of serum TNF-, serum IL-I, serum IL-6, and tissue TBARS.

Declarations

Ethics approval and consent to participate

Approval of the study was obtained from the Institutional Review Board (IRB), Damietta Faculty of Medicine, Al-Azhar University and the research is acceptable according to the guidelines and declaration of Helsinki and our committee standard operating procedure guidelines

Consent for publication

Not applicable.

Availability of data and materials

All data and materials are fully presented in the manuscript.

Competing interests

The authors declare that they have no competing interests.

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Author contributions

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References

Aboutalebi H, Heydari Nasrabadi M, Tajabadi Ebrahimi M, Shabani M, Zahedi F. The Healing Effect of Lactobacillus plantarum Isolated from Iranian Traditional Cheese on Acetic Acid Induced Gastric Ulcer in Rats. RJMS 2010; 17 (77) :7-16 URL: http://rjms.iums.ac.ir/article-1-1533-en.html

AlKreathy HM, Alghamdi MK, Esmat A. Tetramethylpyrazine ameliorates indomethacin-induced gastric ulcer in rats: Impact on oxidative, inflammatory, and angiogenic machineries. Saudi Pharm J. 2020;28:916–926.

Amandeep K., Robin S., Ramica S. and Sunil K. Peptic ulcer: A review on etiology and pathogenesis. Int Res. J. Pharmacy. 2012. 3(6): ISSN 2230:8407.

Azhari H., Underwood F., King J., Coward S., Shah S., Ng S., et al. The global incidence of peptic ulcer disease and its complications at the turn of the 21st century: a systematic review. J. Can. Assoc. Gastroenterol. 2018 (113): 684–685. 10.1093/jcag.gwy009.036

Cano-Garrido O, Seras-Franzoso J, Garcia-Fruitós E. Lactic acid bacteria: Reviewing the potential of a promising delivery live vector for biomedical purposes. Microb Cell Fact. 2015;14:137. doi: 10.1186/s12934-015-0313-6.

Crouzet L, Rigottier-Gois L, Serror P. Potential use of probiotic and commensal bacteria as non-antibiotic strategies against vancomycin-resistant enterococci. FEMS Microbiol Lett. 2015;362:fnv012. doi: 10.1093/femsle/fnv012.

Drury R and Wallington E: Carleton, s Histological Technique. 4th Ed. Oxford. Univ. Press, New York, Toronto. 1980 p p: 115-119.

El-Saka M, Abo Zeid A, Abou Fard G. Effect of L-citrulline and ranitidine on indomethacin-induced gastric ulcer in male albino wister rats. Bull Egypt Soc Physiol Sci. 2014;34:277–292.

Ferreira Dos Santos T, Alves Melo T, Almeida ME, Passos Rezende R, Romano CC. Immunomodulatory Effects of Lactobacillus plantarum Lp62 on Intestinal Epithelial and Mononuclear Cells. Biomed Res Int. 2016;2016:8404156. doi: 10.1155/2016/8404156. Epub 2016 Jun 30. PMID: 27446958; PMCID: PMC4944036.

Fong, S. Y., Wong, Y. C., Xie, C., & Zuo, Z. Herb-drug interactions between Scutellariae Radix and mefenamic acid: Simultaneous investigation of pharmacokinetics, antiinflammatory effect and gastric damage in rats. J. Ethnopharmacol, 2015. (170): 106–116.

Gambero, A., Becker, T. L., Zago, A. S., De Oliveira, A. F., & Pedrazzoli, J. J. R. Comparative study of anti-inflammatory and ulcerogenic activities of different cyclo-oxygenase inhibitors. Inflammopharmacology. 2005 (13): 441–454.

Goderska K., Pena S. A., Alarcon T. Helicobacter pylori treatment: antibiotics or probiotics. Appl. Microbiol. Biotechnol. 2018 (102): 1–7. 10.1007/s00253-017-8535-7

Haghshenas B, Abdullah N, Nami Y, Radiah D, Rosli R, Khosroushahi AY. Different effects of two newly-isolated probiotic Lactobacillus plantarum 15HN and Lactococcus lactis subsp. Lactis 44Lac strains from traditional dairy products on cancer cell lines. Anaerobe. 2014;30:51–59. doi: 10.1016/j.anaerobe.2014.08.009.

Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Canani RB, Flint HJ, Salminen S, et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014;11:506–514. doi: 10.1038/nrgastro.2014.66.

Huang Y, Wang J, Quan G, Wang X, Yang L, Zhong L. Lactobacillus acidophilus ATCC 4356 prevents atherosclerosis via inhibition of intestinal cholesterol absorption in apolipoprotein E-knockout mice. Appl Environ Microbiol. 2014;80:7496–7504. doi: 10.1128/AEM.02926-14.

Imhann F., Bonder M. J., Vila A. V., Fu J., Mujagic Z., Vork L., et al. Proton pump inhibitors affect the gut microbiome. Gut Microb. 2017 (65): 740–748. 10.1136/gutnjnl-2015-310376

Jaldani V, Dezfoulian O, Shahsavari G. Betaine improves gastroprotective effects of ranitidine and omeprazole against indomethacin-induced gastric ulcer in rats. Herb Med J. 2017;2:9–17.

Ji Y., Park S., Park H., Hwang E., Shin H., Pot B., et al. Modulation of active gut microbiota by Lactobacillus rhamnosus GG in a diet induced obesity murine model. Front. Microbiol.2018 (9):710 10.3389/fmicb.2018.00710

Khoder G, Al-Menhali AA, Al-Yassir F, Karam SM. Potential role of probiotics in the management of gastric ulcer. Exp Ther Med. 2016 Jul;12(1):3-17. doi: 10.3892/etm.2016.3293. Epub 2016 Apr 26. PMID: 27347010; PMCID: PMC4906699.

Khoder G., Al-Menhali A. A., Al-Yassir F., Karam S. M. Potential role of probiotics in the management of gastric ulcer. Experim. Therap. Med.2016 (12): 3–17. 10.3892/etm.2016.3293

Ko JS, Yang HR, Chang JY, Seo JK. Lactobacillus plantarum inhibits epithelial barrier dysfunction and interleukin-8 secretion induced by tumor necrosis factor-alpha. World J Gastroenterol. 2007 Apr 7;13(13):1962-5. doi: 10.3748/wjg.v13.i13.1962. PMID: 17461497; PMCID: PMC4146973.

Liu J., Wang F., Luo H., Liu A., Li K., Li C., et al. Protective effect of butyrate against ethanol-induced gastric ulcers in mice by promoting the anti-inflammatory, anti-oxidant and mucosal defense mechanisms. Int. Immunopharmacol. 2016 (30): 179–187. 10.1016/j.intimp.2015.11.018

Marchesi JR, Adams DH, Fava F, Hermes GD, Hirschfield GM, Hold G, Quraishi MN, Kinross J, Smidt H, Tuohy KM, et al. The gut microbiota and host health: A new clinical frontier. Gut. 2016;65:330–339. doi: 10.1136/gutjnl-2015-309990.

Panula, P., Chazot, P. L. and Cowart, M. International Union of Basic and Clinical Pharmacology. XCVIII. Histamine Receptors. Pharmacol Rev. 2015. 67(3): 601–655.

Pearse A : Histochemistry, Theoretical and Applied. 3rd Ed vol I. 1977. Churchill Livingstone, Lond) .

Şenol K., Özkan M. B., Vural S., Tez M. The role of inflammation in gastric cancer. Inflamm. Cancer. 2014: 235–257. 10.1007/978-3-0348

Siddique, O., Ovalle, A., Siddique, A. S., & Moss, S. F. Helicobacter pylori infection: An update for the internist in the age of increasing global antibiotic resistance. Am. J. Med., 2018(131), 473–479

Suleyman H, Albayrak A, Bilici M, Cadirci E, Halici Z. Different mechanisms in formation and prevention of indomethacin-induced gastric ulcers. Inflammation. 2010;33:224–234.

Toljamo K., Niemelä S., Karvonen A. L., Karttunen R., Karttunen T. J. (2011). Histopathology of gastric erosions association with etiological factors and chronicity. Helicobacter. 2011. 16: 444–451. 10.111/j.1523-5378.2011.00871.x

Ugan RA, Un H. The protective roles of butein on indomethacin induced gastric ulcer in mice. Eurasian. J. Med. 2020. (52): 265–270.

Waller D.G., Renwick A.G. and Hillier K. Medical pharmacology and therapeutics, 2nd ed. El Sevier Limited.2005: 347–401.

Wu, Y, Chen, H, Zou, Y, Yi, R, Mu, J, Zhao, X. Lactobacillus plantarum HFY09 alleviates alcohol-induced gastric ulcers in mice via an anti-oxidative mechanism. J Food Biochem. 2021; 45(5):e13726. https://doi.org/10.1111/jfbc.13726

Zhang M, Fan X, Fang B, Zhu C, Zhu J, Ren F. Effects of Lactobacillus salivarius Ren on cancer prevention and intestinal microbiota in 1,2-dimethylhydrazine-induced rat model. J Microbiol. 2015;53:398–405. doi: 10.1007/s12275-015-5046-z.