Food Biotechnology

ISSN: 0890-5436 (Print) 1532-4249 (Online) Journal homepage: www.tandfonline.com/journals/lfbt20

The Gastroprotective Effect of *Lactiplantibacillus plantarum* E2_MCCKT on Cold Restraint Stress-Induced Gastric Ulcer Mice

Tridip K. Das, Subhasree Mal, Titli Panchali, Abhijit Banik, Shrabani Pradhan & Kuntal Ghosh

To cite this article: Tridip K. Das, Subhasree Mal, Titli Panchali, Abhijit Banik, Shrabani Pradhan & Kuntal Ghosh (2025) The Gastroprotective Effect of *Lactiplantibacillus plantarum* E2_MCCKT on Cold Restraint Stress-Induced Gastric Ulcer Mice, Food Biotechnology, 39:2, 144-160, DOI: 10.1080/08905436.2025.2494985

To link to this article: https://doi.org/10.1080/08905436.2025.2494985

FOOD BIOTECHNOLOGY
2025, VOL. 39, NO. 2, 144–160
https://doi.org/10.1080/08905436.2025.2494985

Taylor & Francis
Taylor & Francis Group

The Gastroprotective Effect of *Lactiplantibacillus plantarum* E2_MCCKT on Cold Restraint Stress-Induced Gastric Ulcer Mice

Tridip K. Das[a,b], Subhasree Mal[a,b], Titli Panchali[b,c], Abhijit Banik[d], Shrabani Pradhan[c], and Kuntal Ghosh[a]

[a]Department of Biological Sciences, Midnapore City College, Midnapore, India; [b]Biodiversity and Environmental Studies Research Center, Midnapore City College affiliated to Vidyasagar University, Midnapore, India; [c]Department of Paramedical and Allied Health Sciences, Midnapore City College, Midnapore, India; [d]Department of Physiology, Midnapore College (Autonomous), Midnapore, India

ABSTRACT

In this study, we evaluated the gastroprotective effects of *Lactiplantibacillus plantarum* E2_MCCKT (isolated from fermented food) using a cold-restraint stress-induced gastric ulcer mice model. Male mice (28.4 ± 1.62 g) were divided into three groups ($n = 10$): normal (N), positive control (PC), and treatment with *Lp. plantarum* E2_MCCKT (T) group. After 30 days of oral supplementation (10^9 CFU/day), the treatment group had significantly less gastric erosion. The histopathological study clearly showed that probiotic *Lp. plantarum* E2_MCCKT could protect the stomach epithelial cells from cold injury. The strain also modulated gastric inflammation by upregulating IL-10 (2.31 ± 0.01-fold) and downregulating IFN-γ (2.17 ± 0.03-fold), IFN-λ (10.05 ± 0.03-fold), and IL-12 (1.31 ± 0.03-fold) mRNA expressions in the T group. Additionally, it increased IL-10 (1.06 ± 0.06-fold) and PPAR-α (1.13 ± 0.02-fold) protein expression while reducing IL-6 (1.18 ± 0.07-fold) and PPAR-γ (1.11 ± 0.03-fold), demonstrating its anti-inflammatory properties. Hence, the isolated *Lp. plantarum* E2_MCCKT might be an alternative therapeutic agent for cold-restraint stress-induced gastric ulcer treatment.

KEYWORDS

Probiotic; gastric ulcer; anti-inflammatory; gastroprotective; PPAR-α

Introduction

Gastric ulcers represent a chronic inflammatory condition within the gastrointestinal tract (GI), primarily affecting the stomach. The worldwide prevalence of gastric ulcers in developed countries is about 40% (Ngwa et al. 2022). Currently, a wider number of people in the Indian population have been frequently suffering from this epidemic disease (Banik et al. 2019). Over the past few decades, the research has advanced to offer new insights into the therapeutic and prevention of gastric ulcers (Shah and Patel 2012). It is caused by an imbalance between damaging factors [hydrochloric acid (HCl) and

CONTACT Kuntal Ghosh micro.kuntal@gmail.com; kuntalghosh@mcconline.org.in Department of Biological Sciences, Midnapore City College, Midnapore, West Bengal 721129, India

Supplemental data for this article can be accessed online at https://doi.org/10.1080/08905436.2025.2494985.

pepsin] and protective mechanisms (prostaglandin, bicarbonate, and mucus) within the stomach (Banik et al. 2019). It is usually marked by neutrophil infiltration, various degrees of tissue damage, decreased blood flow, heightened oxidative stress, and inflammation (Ngwa et al. 2022).

Several ulcerogenic factors are involved in gastric ulcer development, including *Helicobacter pylori* infection, nonsteroidal anti-inflammatory drugs [NSAIDs], smoking and alcohol consumption. Moreover, prolonged anxiety, hemorrhage, surgical shock, burns, trauma, and stress are also responsible for ulcer formation. These factors can create an imbalance between aggressive factors (cold temperatures, contact with water, smoking, alcohol consumption, prolonged use of NSAIDs, and psychological stress) and mucosal defensive elements (bicarbonate, prostaglandin, nitric oxide, pepsin, and free radicals) (Komar et al. 2018; Mal et al. 2025; Yang et al. 2021).

Cold restraint stress (CRS) is one of the possible ways to develop gastric ulcers. Exposure to cold acts as a stressor that directly activates the vagus nerve, which extends from the brain to the stomach and plays a crucial role in acid production. The activation of this nerve is a significant factor in the formation of gastric ulcers (Mal et al. 2025). Additionally, cold restraint greatly restricts individuals' physical activity and significantly diminished the stomach's capacity for absorption. Notably, restraint at 4°C led to a marked increase in the ulcer index. Moreover, the mucous layer erosion may result from the increased acid and pepsin secretion by parietal and zymogenic cells. Once the mucous layer is damaged, the surface epithelium can peel away, exposing the endothelial cells of the capillaries in the underlying connective tissue. This cascade of events ultimately leads to necrosis of epithelia and connective tissue, culminating in ulcer formation (Khoder et al. 2016; Tarnawski, Ahluwalia, and Jones 2013).

Gastric ulcer treatment is prolonged compared to duodenal ulcers and has a higher chance of leading to oncogenesis (Chen et al. 2018). If left untreated, this condition may result in perforation and bleeding, causing severe complications that could progress to gastric cancer and ultimately lead to mortality (Yang et al. 2021). Currently, numerous anti-ulcer drugs (omeprazole, lansoprazole, ranitidine, famotidine, pirenzepine, and telezipine) are available as treatments. The efficacy of these drugs in combating ulcers may be attributed to their ability to fortify the protective barriers of the gastric mucosa through several mechanisms, including antioxidant effects (Laprasert et al. 2020), anti-inflammatory properties (Aboul Naser et al. 2020), and stimulation of mucus secretion (Moawad et al. 2019). These drugs could care for gastric ulcers but cannot prevent ulcer recurrences (Shah and Patel 2012). Therefore, the drugs and therapeutic approaches are ineffective for noninfectious gastric ulcer management (Moawad et al. 2019). Some adverse effects and lower efficacies of the drugs mentioned above are gastrointestinal reactions, rebound effects,

hypergastrinemia, hepatorenal toxicity, headaches, nausea, impotence, and arrhythmia (Jin et al. 2022; Kim 2021; Kuna et al. 2019). These medication offer temporary pain relief but does not address the root cause of gastric ulcers. Hence, it is crucial to prioritize the development of safe and highly effective therapy for gastric ulcer management.

According to the Joint FAO/WHO Working Group (2002), "Probiotics are non-pathogenic live microorganisms that provide positive health benefits to the host when consumed in adequate amounts." Probiotics have emerged as a promising alternative strategy for preventing and treating various intestinal inflammatory disorders, notably ulcerative colitis (UC) (Das et al. 2022; Senol et al. 2011). A mechanistic study indicated that probiotics influenced protein expression in the gastric wall cells, resulting in an increased production of new blood cells, which contributed to the healing of ulcers (Singh, Deol, and Kaur 2012). Additionally, probiotics produce short-chain fatty acids that are vital nutrients for the cells lining of the gut, helping to improve blood flow to the intestinal wall. *Lactobacillus* plays a significant role in enhancing the immune system by stimulating immune cells, thereby reinforcing the body's defense mechanisms. Several studies reported that *Lactobacillus plantarum* strains HFY09, KSFY06, and ZS62 could significantly reduce the extent of gastric ulceration induced by either ethanol or hydrochloric acid (Li et al. 2020; Wang et al. 2020a; Wu et al. 2021). These strains could enhance the gastric mucosal defensive factors and bolster antioxidant capacity while simultaneously inhibiting inflammatory processes. In another study on acute gastric damage caused by intragastric administration of ethanol in rats, pre-treatment with the probiotic *Lactobacillus rhamnosus* GG (LGG) strain was found to increase the basal level of prostaglandin E2 (PGE2) in the gastric mucosa (Sun et al. 2018). Additionally, probiotics might promote the expression of mucin genes in colonic epithelial cell lines and could enhance the mucus-secreting layer in the gastric mucosa of rats (Sun et al. 2018). Probiotics are capable of modifying the expression of inflammatory cytokines within the body, thereby mitigating the severity of inflammation (Yu et al. 2020). The effectiveness of probiotics largely depends on the specific strain used. However, studies suggested that probiotics could be used as an essential therapeutic agent for a noninfectious gastric ulcer caused by acid, alcohol, stress, and drugs (Sun et al. 2018; Yang et al. 2021). The effects of probiotic treatment on acute gastric damage remain largely underexplored. This lack of research can be attributed to the challenging physiological conditions present in the gut, including acidic pH levels, digestive enzymes, bile acids, and mechanical stress, which can hinder the survival and proliferation of probiotic microorganisms. Nevertheless, administering sufficient probiotics may mitigate these challenges and enhance their effectiveness in gastrointestinal health.

In this current investigation, we explore the anti-gastric ulcer activity of the *Laciplantibacillus plantarum* E2_MCCKT strain. It has already been

established that our isolated *Lp. plantarum* E2_MCCKT strain from the traditional fermented rice beverage (haria) exhibited *in vitro* probiotic activities (Das et al. 2024). The bacterium demonstrated high survivability in acidic conditions (pH 2 and 3) and in 2% bile salt. Additionally, it displayed moderate auto aggregation (52.52%) and hydrophobicity (38.08%) (Das et al. 2024). In this study, a cold-induced gastric ulcer model was established to inspect the mechanism and actions of the *Lp. plantarum* E2_MCCKT strain on the gastric mucosa of CRS-induced ulcer mice.

Materials and methods

Chemicals

All chemicals used were procured from Hi-Media Laboratories, India, except for Folin-Ciocalteu Reagent, N, N, N′, N′-Tetramethylethylenediamine (TEMED), acrylamide, bis-acrylamide, bovine serum albumin, and ammonium persulfate, which were obtained from Sisco Research Laboratories, India.

Lactiplantibacillus plantarum E2_MCCKT preparation

The viable plate count method was employed to calculate colony-forming units (CFU/mL) of *Lp. plantarum* E2_MCCKT strain for oral administration to the mice. The bacterial strain was cultivated in Rogosa SL broth (Hi-media, India) and incubated in anaerobic conditions (5%, CO_2) at 37°C for 24 h. The bacterial cells were collected by centrifugation at 8,000 rpm for 5 min and washed twice with sterilized 0.2 M PBS (pH-7.2), followed by dissolving in sterilized normal saline containing 2% sucrose for oral gavaging. The normal (N) and positive control (PC) groups received 20 µL of normal saline containing 2% sucrose for 30 days.

Animal grouping and care

The Animal Ethical Committee of Midnapore City College (MCC/IAEC-KG /10/23–004) granted approval for the animal experiments. In this study, we used BALB/c male albino mice, and each mice had an average body weight of 28.4 ± 1.62 g. Before the experiment, all mice were acclimatized for 14 days in standardized laboratory conditions (32 ± 2°C and 50% moisture) with a 12-h:12 h light/dark cycle. The mice were provided with normal food and had free access to water *ad libitum*. The normal food consisted of carbohydrates, protein, and fat at a respective ratio of 64.2%, 22.3%, and 13.5%. Afterward, the mice were randomly picked up and distinguished into three different groups: normal (N) group, positive control (PC) group, and treatment (T) group. Each group contain 10 numbers of mice ($n = 10$). Gastric ulcer was induced in PC

group mice and T group mice after 30 days by CRS for 3 h by keeping the mice at 4°C. The T group was provided pre-supplementation of *Lp. plantarum* E2_MCCKT strain with 10^9 CFU/day (20 µL contained 10^9 CFU) using a rounded/bulb-tipped gavage needle continuously for 30 days. The N and PC group received 20 µL of normal saline containing 2% sucrose for 30 days. The last dose was given 30 min before CRS induction. Based on numerous studies, it has been demonstrated that an intake of 10^9 CFU of probiotic bacteria per day is an effective dosage for managing gastric ulcers (Ngwa et al. 2022; Park et al. 2020; Suo et al. 2016).

Induction of gastric ulceration

Mice were kept in a fasting condition with available access to water *ad-libitum* for 24 h to avoid coprophagia. Thereafter, all the PC and T group mice were kept in individual boxes in an immobilization condition. The boxes were positioned in a refrigerator at 4°C for 3 h. Research indicated that the concomitant application of body restraint protocols and exposure to hypothermic conditions significantly accelerated the onset of gastric ulceration, with effects becoming evident within a reduced timeframe of just 3 h (Landeira-Fernandez 2004). Simultaneously, the N group was restrained at room temperature for a similar duration. To follow up on the experiment, the refrigerator door was opened at every 30 min for inspection and to avoid possible regulators of stomach function, all the procedures were continued at the same time of the day (Ibrahim 2013).

Macroscopic examination of gastric mucosa and ulcer index (UI) assessment

The stomach in each case was carefully positioned in the dissection tray, and any blood residue was eliminated using a solution of 0.9% normal saline. Afterward, stomach mucosal erosion was confirmed through a thorough macroscopic examination. The degree of mucosal erosion within three distinct groups was assessed using the following scoring system: absence of ulceration = 0; minor ulceration (1–2 mm) = 1; moderate ulceration (3–4 mm) = 2; extensive ulceration (5–6 mm) = 4; and extensive ulceration (>6 mm) = 8 (Banik et al. 2019).

UI = Σ Total ulcer score of individual mice group/Total number of mice in a group.

The calculation of ulcer inhibition percentage concerning the positive control group was
performed using the following formula:

$$\text{Inhibition (\%)} = (\text{Uc-Ut})/\text{Uc} \times 100$$

Where Uc is denoted as a positive control (PC) group, and Ut is expressed as a treated (T) group.

Histopathological examination of stomach tissues

A small portion (5 µm) of the collected stomach was then fixed with 10% formalin. Thereafter, hematoxylin and eosin (H&E) staining was performed by immediate processing of paraffin-embedded technique to determine the histopathological changes with the help of a photomicroscope (4X, magnification; Olympus, Tokyo 163–0914, Japan) (Sun et al. 2018).

Evaluation of mRNA expression using semi-quantitative polymerase chain reaction (PCR) study

The total mRNA expression in the stomach tissue was assessed following a standard RNA isolation procedure. Subsequently, cDNA was synthesized using the Hi-media cDNA synthesis kit for PCR amplification. A PCR amplification was then carried out, utilizing the cDNA as a template and multiple gene-specific primers with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the housekeeping gene (Supplementary Table S1). The standard PCR amplification consisted of 40 cycles, commencing with an initial step at 95°C for 4 min, followed by cycles of denaturation at 95°C for 30 sec, annealing at 55–60°C for 45 sec, and extension at 72°C for 50 sec, culminating in a final elongation step lasting 7 min at 72°C. After the completion of PCR amplification, the concentrations of various cytokines [interferon gamma (IFN-γ), interferon lambda (IFN-λ), interleukin-12 (IL-12), interleukin-10 (IL-10), and GAPDH)] were quantified using a GS700 Imaging Densitometer and Molecular Analyst software (Version 1.5; Bio-Rad Laboratories, CA, USA) (Banik et al. 2019).

Quantitative Real-Time Reverse Transcription Polymerase Chain Reaction (qRT-PCR) for mRNA expression study

Again, previously synthesized cDNA was analyzed by using qPCR (G8830A; AriaMx Real-Time PCR; Agilent Technologies, India). The reaction mixture was prepared using 1 µL of cDNA as a template, along with 10 µL of 2X SYBR-Green Mixture (Agilent Technologies, India) containing gene-specific primers (Supplementary Table S1) and making the final volume up to 20 µL. The qPCR process included 40 cycles: denaturation at 95°C for 30 sec, annealing at 55°C–60°C for 1 min, and extension at 72°C for 30 sec. The fluorescence intensity threshold for all samples was manually established and measured at

the end of each cycle. This threshold cycle, denoting the highest fluorescence intensity point during the exponential phase of PCR amplification [quantification cycle (Cq)], was employed for the relative quantification of the target genes, comparing their Cqs to that of GAPDH (housekeeping gene) at a consistent fluorescence rate. The $2^{-\Delta\Delta CT}$ method was employed to determine the relative fold change of the target genes (Jana et al. 2023; Livak and Schmittgen 2001).

Evaluation of inflammatory and anti-inflammatory cytokines expression

The stomach tissues were homogenized with ice-cold radioimmunoprecipitation assay (RIPA) buffer [Tris-HCL 50 mM, NaCl 150 mM, ethylenediamine tetraacetic acid (EDTA) 5 mM, and 1% Titron-X100 and cocktail protease inhibitors, Hi-media] and the supernatant was collected by following 2 min centrifugation at 10,000 rpm. Later, the Lowry method was used for protein quantification. In brief, 1 mL of 1 N sodium hydroxide (NaOH) was heated in a test tube for a few sec. Afterward, 1 mL of the sample was added and incubated for 3–4 minutes. Next, 5 mL of alkaline copper (II) sulfate ($CuSO_4$) and sodium potassium tartrate ($C_4H_4KNaO_6{\cdot}4\,H_2O$) were added and incubated at 37 °C for 10 min. Finally, 0.5 mL of Folin-Ciocalteu reagent was mixed, resulting in a blue-green color after 30 min of incubation, detected at $OD_{750\,nm}$. A 50-µg protein sample was placed in a loading buffer and boiled for 5 min. After that, the sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was completed, followed by blotting using a nitrocellulose membrane (BioRad, China). Subsequently, the membrane was subjected to an overnight incubation at 4°C with primary antibodies after being pre-blocked with 5% bovine serum albumin (BSA). The primary antibodies employed in this study encompassed β-actin (Abgenex, India), peroxisome proliferator-activated receptor-alpha (PPAR-α), peroxisome proliferator-activated receptor gamma (PPAR-γ), interleukin-6 (IL-6), and IL-10 (Santa Cruz Biotechnology, USA). Following this, the membrane underwent a 1-h incubation with horseradish peroxidase (HRP) -conjugated anti-mouse secondary antibody. The exact protein bands were detected by using 3,3′-Diaminobenzidine (DAB) (Das et al. 2024).

Statistical analysis

Each dataset was generated through three repetitions and reported with the Standard Error of the Mean (SEM). Statistical significance was determined at the $p < 0.05$ levels. The data underwent a one-way ANOVA analysis followed by Tukey's post-hoc analysis, utilizing Sigma Plot 12.5 software, USA.

Results

Anti-gastric ulcer activities of Lactiplantibacillus plantarum E2_MCCKT

Macroscopic analysis of stomach

Following a macroscopic examination of stomach tissue after 3 h of cold-restraint stress (CRS) induction, it was observed that the PC group exhibited extensive damage to the gastric mucosa, along with visible hemorrhagic necrosis, in comparison to the N group (Fig. 1a,b). However, the mice pre-treated with *Lp. plantarum* E2_MCCKT had moderate to mild gastric injuries and less hemorrhagic necrosis occurred in the gastric mucosa (Fig. 1c).

Histopathological examination of stomach tissue

The stomach tissue cells of the normal mice (N) group were tightly arranged without being dislodged, with complete structure, and the glands were clearly visible (Fig. 2a). Conversely, in the ulcer control (PC) group, we observed extensive damage to the gastric mucosa, marked necrosis, and severe hemorrhagic lesions within the glandular region of the stomach (Fig. 2b). The stomach ulcer varied from small pinpoints to lengthened erosions ranging from 1 to 8 mm/0.5–1.5 mm (l/w). The ulcer index (UI) in the untreated ulcer control group significantly increased (12.66 ± 1.25) after 3 h exposure to CRS (Table 1). On the other hand, the probiotic treatment (T) group clearly showed

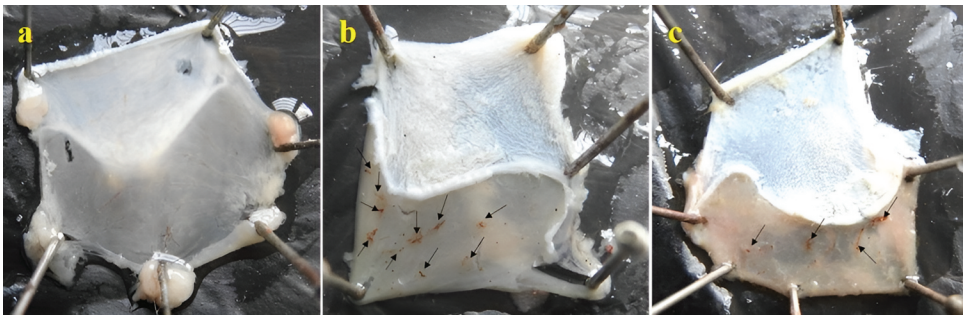

Figure 1. Macroscopic view of the stomach tissues after cold-restraint stress-induced ulcer in different experimental mice ($n = 10$); (a) Normal (N) Group, (b) Positive control (PC) Group, and (c) Treatment (T) Group.

Table 1. Anti-ulcerogenic effect of *Lp. plantarum* E2_MCCKT.

Group	Ulcer Index (UI)	Percentage of Inhibition
Normal (N)	0.0 ± 0.0^{c}	–
Positive control (PC)	12.66 ± 1.25^{a}	–
Treatment (T)	4.20 ± 1.12^{b}	67.17%

Each value represents the mean ($n = 10$). N = normal group; PC = positive control group; T = treatment group (*Lp. plantarum* E2_MCCKT). The values with different superscript letter are significantly different at ($p < 0.05$) levels.

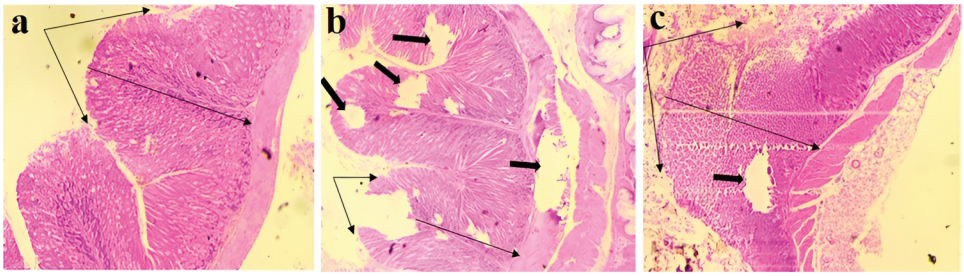

Figure 2. Histopathological view of the stomach tissue after cold-restraint stress-induced ulcer in different experimental mice ($n = 10$); (a) Normal (N) group, (b) Positive control (PC) group, (c) Treatment (T) group (Sections are H&E stained). The observation was made under 4X magnification.

that the gastric tissue cells were loosely arranged, blood vessels were dilated, and inflammatory cell infiltration was noted. However, the pre-treatment of *Lp. plantarum* E2_MCCKT markedly reduced the gastric erosion in the stomach mucosa (4.20 ± 1.12), as compared to the ulcer control (PC) group, and the ulcer inhibition percentage was 67.17% (Table 1). Consequently, gastric mucosa started repairing activity like normal gastric mucosa (Fig. 2c).

Gene expression study

The effectiveness of *Lp. plantarum* E2_MCCKT on ulcer-related genes of the stomach were investigated (Suplementary Figure S1). The gene expression levels of IFN-γ (1.79 ± 0.05-fold), IFN-λ (5.10 ± 0.09-fold), and IL-12 (2.28 ± 0.02-fold) were significantly upregulated in the ulcer control (PC) group due to CRS induction when compared to the normal (N) group (Fig. 3a–c). Conversely, after the prior supplementation of probiotic *Lp. plantarum* E2_MCCKT significantly increased the anti-inflammatory cytokine IL-10 (2.31 ± 0.01-fold) (Fig. 3d) in connection with the downregulation of pro-inflammatory cytokines such as IFN-γ (2.17 ± 0.03-fold), IFN-λ (10.05 ± 0.03-fold), and IL-12 (1.31 ± 0.03-fold) in the gastric mucosa of the T group when compared to the PC group (Fig. 3).

Expression of inflammatory and anti-inflammatory cytokines in gastric tissue

The protein expression related to stomach tissue inflammation, such as PPAR-α, PPAR-γ, IL-6, and IL-10, was analyzed and β-actin was used as an internal standard. The expression of PPAR-α and IL-10 were significantly upregulated (1.13 ± 0.02-fold, and 1.06 ± 0.06-fold) in the T group compared to the PC group (Fig. 4a,d), whereas PPAR-γ (1.11 ± 0.03-fold) and IL-6 (1.18 ± 0.07-

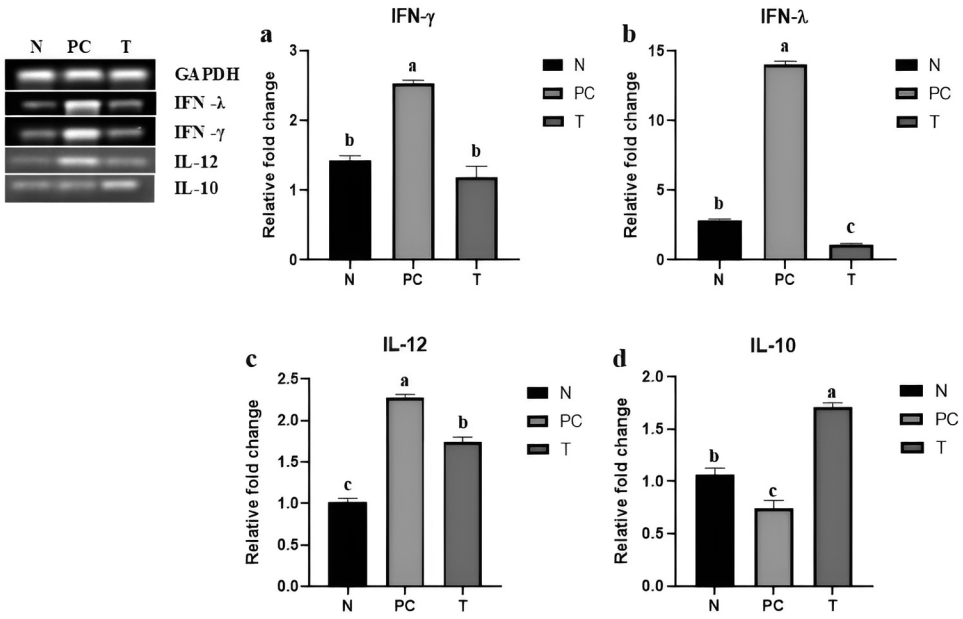

Figure 3. Relative mRNA expression (fold change) of pro- and anti-inflammatory related cytokine marker genes in the stomach tissue of cold restraint stress-induced experimental mice. IFN-γ (a), IFN-λ (b), IL-12 (c) and IL-10 (d). Data with different superscript letters are significantly different at $p < 0.05$ levels. Bars represent the mean ± SE, N=Normal group; PC= positive control group; *T*= treatment group.

fold) expressions were markedly decreased in the T group compared to the PC group (Fig. 4b,c).

Discussion

The pathophysiology of stress-induced stomach ulceration in experimental animals (such as rats and mice) has been extensively researched, but its etiology is still unknown. Therefore, the present study demonstrated the anti-gastric ulcer activities of our isolated *Lp. plantarum* E2_MCCKT strain on the cold-restraint stress (CRS) induced mice model. After 3 h of CRS induction, the mice were anesthetized, and the stomachs of all the mice were collected by a successful dissection. It is widely accepted that stress can cause alterations in gastric acid secretion and motility, leading to varying degrees of ulceration in the gastrointestinal mucosa. The macroscopy and histopathological examination by densitometric analysis showed that the pre-supplementation of *Lp. plantarum* E2_MCCKT significantly reduced the margin of gastric erosion in the stomach of the T group (4.20 ± 1.12). In contrast, the PC group (12.66 ± 1.25) showed a wide margin of erosion and festering in the lining of the stomach mucosa compared to the N group (0.0 ± 0.0). The E2_MCCKT strain or its metabolites might reduce the apoptosis-to-proliferation ratio of

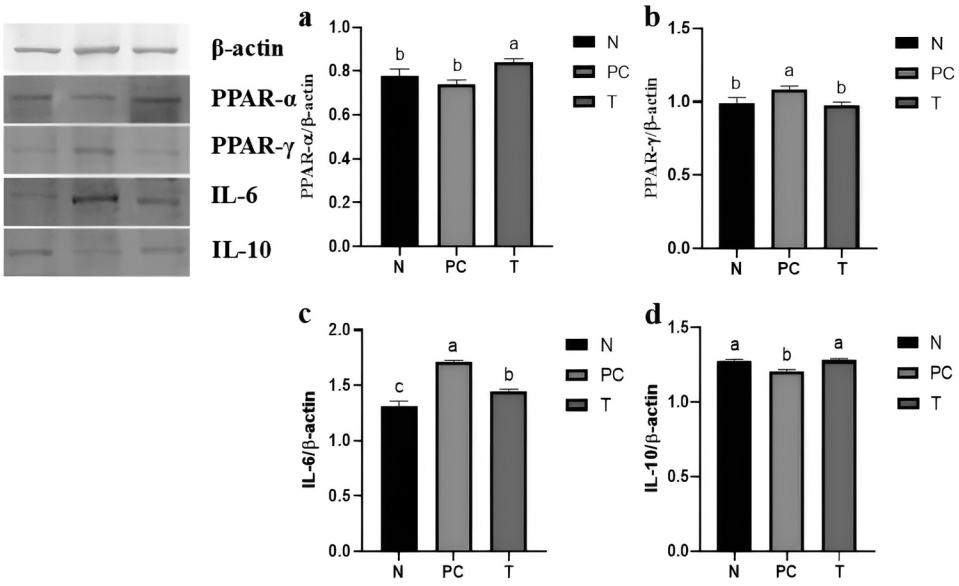

Figure 4. Analysis of inflammatory and anti-inflammatory protein expression; PPAR-α (a), PPAR-γ (b), IL-6 (c) IL-10 (d) in gastric tissue compared to control protein expression (β-actin). Data with different superscript letters are significantly different at $p < 0.05$ levels. Values are expressed as mean ± SE.

cells at the ulcer state as suggested by Lam et al. (2007). This demonstrated a promising enhancement of adequate blood supply that might help in tissue repair and epithelial regeneration. This change reflected an increased cellular turnover rate, which might play a vital role in effectively replacing damaged mucosa. A study revealed that soy-skim milk fermented *L. paracasei* subsp. *paracasei* NTU101 and *L. plantarum* NTU 102 could significantly alleviate gastric injury and reduce the ulcer index of the total mucosal injury in a rat model (Liu et al. 2009). Similarly, *L. reuteri* F-9-35, *L. bifidobacterium* BF-1, and *L. rhamnosus* GG could enhance gastric secretion and prevent the downfall of the mucus barrier that helped to increase the recovery rate of the epithelial cells (Gomi et al. 2013; Sun et al. 2018). The transit through the stomach lasts from 5 min to 2 h, during which prolonged exposure to acidic environments poses a challenge for probiotics. Our previous study showed that the isolated bacterium could survive in acidic pH (2 to 3) for 2 h without a notable decrease in numbers (Das et al. 2024). This acid-resistant nature allowed the probiotics to endure in the stomach long enough to provide beneficial effects, even without permanent colonization (Yao et al. 2020). In connection to this, Lam et al. (2007) suggested that this might be attributed to the gastric colonization of the probiotic *Lactobacillus* could stimulate the secretion of prostaglandin E2 (PGE2) from gastric glandular cells, which led to gastric recovery through the production of Mucin-6 (MUC-6), maintaining mucus thickness, and promoting ulcer healing.

Later, we analyzed the inflammation-related genes during CRS-induced gastric ulcers and stomach tissue deprivation. Stress triggers the production of inflammatory cytokines, leading to the infiltration of neutrophils and subsequent damage to the gastric mucosa (Sun et al. 2018). The gene expression levels of IFN-γ, IFN-λ, and IL-12 showed an increase of 1.79-fold, 5.10-fold, and 2.28-fold, respectively, in the PC group of mice, when compared to the N group of mice (Fig. 3a–c). In contrast, pre-supplementation of *Lp. plantarum* E2_MCCKT, the expression of IFN-γ, IFN-λ, and IL-12 were significantly down-regulated by 2.17-fold, 10.05-fold, and 1.31-fold, respectively, and accompanied by a notable upregulation of IL-10 by 2.31-fold in the T group when compared to the PC group (Fig. 3a–d). The elevated level of IL-10 has been found to inhibit the activation of macrophages by suppressing the production of T-helper 1 (Th1) and Th2 cytokines, including IFN-γ, IFN-λ, and IL-12. The probiotic strain E2_MCCKT might have the ability to inhibit cytokine production associated with Th1 and Th2 responses. This suggests that the strain could play a significant role in modulating immune responses by curbing the activity of these two distinct types of helper T cells and reducing gastric inflammation. Numerous studies demonstrated the probiotic bacteria's anti-inflammatory properties in stress-induced ulcers (Senol et al. 2011; Virchenko et al. 2015). Wang et al. (2020b) reported that pretreatment of *L. fermentum* XY18 could decrease the expression of the pro-inflammatory cytokine such as TNF-α, IFN-γ, IL-12, and IL-6 levels and prevent oxidative tissue damage during HCL/Ethanol-induced gastric injury. Moreover, Wu et al. (2021) also reported that *L. plantarum* HFY09 could decrease pro-inflammatory factors such as TNF-α, IL-6, and IL-1β while increasing the anti-inflammatory factor IL-10, thus inhibiting the inflammatory reaction in gastric injury. Concomitantly, our study observed similar findings and agreed with this previous study. Clearly, *Lp. plantarum* E2_MCCKT protected the gastric tissue from erosion and decreased the production of inflammatory cytokines related to gastric ulcers.

We also investigated tissue inflammation by measuring the relative protein expression of the stomach tissue. It has been reported that numerous inflammatory cytokines are generated under the influence of various harmful factors, leading to the infiltration of neutrophils and consequent injuries in the gastric mucosa (Arab et al. 2015). After CRS-induced gastric ulcer, the PC group mice had higher expression of PPAR-γ and IL-6 (1.09-fold and 1.30-fold) compared to the N group (Fig. 4b,c), while PPAR-α and IL-10, anti-inflammatory cytokines were downregulated in the PC group (Fig. 4a,d). The pre-supplementation of probiotic *Lp. plantarum* E2_MCCKT significantly down-regulated the PPAR-γ and IL-6 (1.11-fold, and 1.18-fold) expression, while the expressions of PPAR-α (1.13-fold) and IL-10 (1.06-fold) were increased compared to the PC group (Fig. 4). Therefore, *Lp. planatrum* E2_MCCKT or its metabolites might increase anti-inflammatory cytokine expression (PPAR-α

and IL-10), blocking the activated macrophage and reducing proinflammatory cytokines (PPAR-γ and IL-6) expression. Hence, it indicated that *Lp. plantarum* E2_MCCKT might exhibit anti-inflammatory activity and gastroprotective effects during CRS-induced gastric ulcers (Fig. 5).

The current research highlighted the promising advantages and future applications of the probiotic *Lp. plantarum* E2_MCCKT, particularly in the

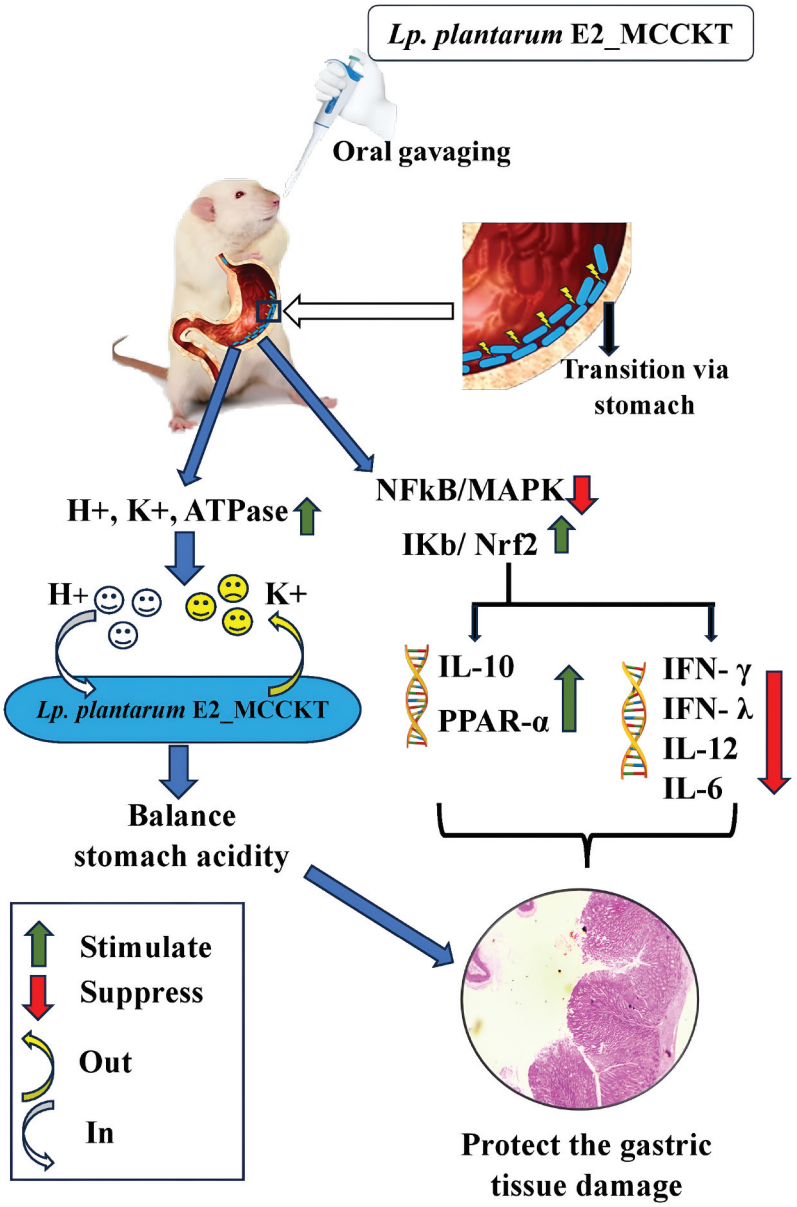

Figure 5. A hypothetical mechanism and action of probiotic *Lp. plantarum* E2_MCCKT on cold restraint stress-induced gastric ulcer management.

treatment of gastric ulcers induced by CRS. As suggested earlier, the probiotic bacteria exhibited different health-beneficial effects (Das et al. 2022); it is likely that *Lp. plantarum* E2_MCCKT could manage the other lifestyle related diseases along with the gastric ulcer amelioration. Thus, the isolated strain might exhibit positive health benefits to the consumers and can be used as an alternative safe therapeutic agent for the management of gastric ulcers. However, it is essential to enhance research initiatives that explore the underlying mechanisms of stress-induced pathophysiology. Additionally, investigating the innovative ways in which probiotics exert their healing effects could open new avenues for effective treatment strategies.

Conclusion

In the present study, we established a CRS-induced ulcer mice model to investigate the anti-ulcer activity of *Lp. planatrum* E2_MCCKT. *Lp. planatrum* E2_MCCKT exhibited gastroprotective activity by increasing the renewal rate of epithelial cells. Moreover, *Lp. planatrum* E2_MCCKT supplementation could increase anti-inflammatory cytokines and minimize gastric inflammation. Therefore, *Lp. planatrum* E2_MCCKT could be used as a potential therapeutic approach for CRS treatment.

Acknowledgments

The authors are thankful to Dr. Pradip Ghosh, the Director of Midnapore City College, for providing the necessary facility. The author TKD is thankful to Morine Human Resource Development Organization for financial assistance.

Disclosure statement

No potential conflict of interest was reported by the author(s).

ORCID

Tridip K. Das http://orcid.org/0000-0002-0080-1858
Subhasree Mal http://orcid.org/0009-0002-9625-1471
Abhijit Banik http://orcid.org/0000-0002-7145-6684
Kuntal Ghosh http://orcid.org/0000-0003-4423-2471

References

Aboul Naser, A., E. Younis, A. El-Feky, M. Elbatanony, and M. Hamed. 2020. Management of Citrus sinensis peels for protection and treatment against gastric ulcer induced by ethanol in rats. *Biomark* 25 (4):349–359. doi: 10.1080/1354750X.2020.1759693.

Arab, H. H., S. A. Salama, H. A. Omar, E. S. A. Arafa, and I. A. Maghrabi. 2015. Diosmin protects against ethanol-induced gastric injury in rats: Novel anti-ulcer actions. *PLOS ONE* 10 (3):0122417. doi: 10.1371/journal.pone.0122417.

Banik, A., J. Mondal, S. Rakshit, K. Ghosh, S. P. Sha, S. K. Halder, C. Ghosh, and K. C. Mondal. 2019. Amelioration of cold-induced gastric injury by a yeast probiotic isolated from traditional fermented foods. *J. Funct. Foods* 59:164–173. doi: 10.1016/j.jff.2019.05.039.

Chen, X., C. Xia, Q. Li, L. Jin, L. Zheng, and Z. Wu. 2018. Comparisons between bacterial communities in mucosa in patients with gastric antrum ulcer and a duodenal ulcer. *Front. Cell. Infect. Microbiol.* 8:126. doi: 10.3389/fcimb.2018.00126.

Das, T. K., P. Kar, T. Panchali, A. Khatun, A. Dutta, S. Ghosh, S. Chakrabarti, S. Pradhan, K. C. Mondal, and K. Ghosh. 2024. Anti-obesity potentiality of *Lactiplantibacillus plantarum* E2_MCCKT isolated from a fermented beverage, haria: A high fat diet-induced obese mice model study. *World J. Microbiol. Biotechnol.* 40 (6):168. doi: 10.1007/s11274-024-03983-3.

Das, T. K., S. Pradhan, S. Chakrabarti, K. C. Mondal, and K. Ghosh. 2022. Current status of probiotic and related health benefits. *Appl. Food Res.* 2 (2):100185. doi: 10.1016/j.afres.2022.100185.

Gomi, A., N. Harima-Mizusawa, H. Shibahara-Sone, M. Kano, K. Miyazaki, and F. Ishikawa. 2013. Effect of *Bifidobacterium bifidum* BF-1 on gastric protection and mucin production in an acute gastric injury rat model. *J. Dairy Sci.* 96 (2):832–837. doi: 10.3168/jds.2012-5950.

Ibrahim, M. 2013. Attenuation of cold restraint stress-induced gastric lesions by sildenafil in rats. *Med. J. Cairo. Univ.* 81:229–233.

Jana, S., P. Mitra, A. Dutta, A. Khatun, T. K. Das, S. Pradhan, D. K. Nandi, and S. Roy. 2023. Early diagnostic biomarkers for acute kidney injury using cisplatin-induced nephrotoxicity in rat model. *Curr. Res. Toxicol.* 5:100135. doi: 10.1016/j.crtox.2023.100135.

Jin, Y., M. Zhang, Y. Wang, Y. Lu, T. Liu, G. Yang, S. Song, and W. Liu. 2022. Protective effect and potential mechanism of the traditional Chinese medicine Shaoyao-Gancao decoction on Ethanol-Induced gastric ulcers in rats. *Evidence-Based Compl. Altern. Med.* 2022 (1):3069089. doi: 10.1155/2022/3069089.

Joint FAO/WHO Working Group, and Joint FAO/WHO Working Group. 2002. Guidelines for the evaluation of probiotics in food. London: World Health Organization, Canada: Food and Agriculture Organization.

Khoder, G., A. A. Al-Menhali, F. Al-Yassir, and S. M. Karam. 2016. Potential role of probiotics in the management of gastric ulcer. *Exp. Ther. Med* 12 (1):3–17. doi: 10.3892/etm.2016.3293.

Kim, G. H. 2021. Proton pump inhibitor-related gastric mucosal changes. *Gut. Liver* 15 (5):646. doi: 10.5009/gnl20036.

Komar, O. M., N. M. Kizlova, O. D. Trylevych, and V. V. Kravchenko. 2018. Risk factors for adverse course of gastric and duodenal peptic ulcer. *Wiad. Lek. (Warsaw, Poland: 1960)* 71 (1 pt 2):160–164.

Kuna, L., J. Jakab, R. Smolic, N. Raguz-Lucic, A. Vcev, and M. Smolic. 2019. Peptic ulcer disease: A brief review of conventional therapy and herbal treatment options. *J. Clin. Med.* 8 (2):179. doi: 10.3390/jcm8020179.

Lam, E. K., L. Yu, H. P. Wong, W. K. Wu, V. Y. Shin, E. K. Tai, W. H. So, P. C. Woo, and C. H. Cho. 2007. Probiotic *Lactobacillus rhamnosus* GG enhances gastric ulcer healing in rats. *Eur. J. Pharmacol* 565 (1–3):171–179. doi: 10.1016/j.ejphar.2007.02.050.

Landeira-Fernandez, J. 2004. Analysis of the cold-water restraint procedure in gastric ulceration and body temperature. *Physiol. & Behav* 82 (5):827–833. doi: 10.1016/j.physbeh.2004.06.016.

Laprasert, C., P. Kunanusorn, A. Panthong, P. Khonsung, N. Chiranthanut, and C. Rujjanawate. 2020. Gastric ulcer healing activity against acidified ethanol-induced gastric

ulcer and gastroprotective mechanisms of Zingiber simaoense rhizome ethanol extract in rats. *Pharm. Mag* 16 (68):152–160. doi: 10.4103/pm.pm_389_19.

Li, F., H. Sun, G. Ran, X. Liu, R. Yi, F. Tan, X. Zhao, and H. Liu. 2020. Preventive effect of *Lactobacillus plantarum* HFY09 on HCl/ethanol-induced gastric injury in mice. *Appl. Biol. Chem.* 63 (1):1–11. doi: 10.1186/s13765-020-00536-8.

Liu, C. F., C. L. Hu, S. S. Chiang, K. C. Tseng, R. C. Yu, and T. M. Pan. 2009. Beneficial preventive effects of gastric mucosal lesion for soy– skim milk fermented by lactic acid bacteria. *J. Agric. Food. Chem* 57 (10):4433–4438. doi: 10.1021/jf900465c.

Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. *Methods* 25 (4):402–408. doi: 10.1006/meth.2001.1262.

Mal, S., T. K. Das, S. Pradhan, and K. Ghosh. 2025. Probiotics as a therapeutic approach for non-infectious gastric ulcer management: A comprehensive review. *Probiotics. Antimicrob Proteins* 17 :393–418. doi: 10.1007/s12602-024-10348-7.

Moawad, H., S. A. El Awdan, N. A. Sallam, W. I. El-Eraky, and M. A. Alkhawlani. 2019. Gastroprotective effect of cilostazol against ethanol- and pylorus ligation–induced gastric lesions in rats. *Naunyn Schmiedebergs Arch. Pharmacol.* 392 (12):1605–1616. doi: 10.1007/s00210-019-01699-y.

Ngwa, F. A., B. T. Fossi, G. S. Taiwe, L. L. T. Tatsinkou, P. T. Bobga, M. T. Ndalloka, D. D. Lea, B. Y. Wanyu, and S. Wanji. 2022. Isolation, characterisation and evaluation of antiulcerogenic potentials of probiotic lactic acid bacteria isolated from fermented milk and palm wine against ethanol-induced gastric ulcer in mice. *J. Biosci. Med. (Irvine)* 10 (9):311–331. doi: 10.4236/jbm.2022.109022.

Park, H., D. Cho, E. Huang, J. Y. Seo, W. G. Kim, S. D. Todorov, Y. Ji, and W. H. Holzapfel. 2020. Amelioration of alcohol-induced gastric ulcers through the administration of *Lactobacillus plantarum* APSulloc 331261 isolated from green tea. *Front Microbiol.* 11:420. doi: 10.3389/fmicb.2020.00420.

Senol, A., M. Isler, A. G. Karahan, G. B. Kilic, H. Kuleasan, S. Kaya, M. Keskin, I. Goren, U. Saritas, B. C. Aridogan, et al. 2011. Preventive effect of probiotics and α-tocopherol on ethanol-induced gastric mucosal injury in rats. *J. Med. Food* 14 (1–2):173–179. doi: 10.1089/jmf.2010.0040.

Shah, J. S., and J. R. Patel. 2012. Anti-ulcer activity of Lucer against experimentally induced gastric ulcers in rats. *AYU* 33 (2):314–316. doi: 10.4103/0974-8520.105260.

Singh, P. K., P. K. Deol, and I. P. Kaur. 2012. Entrapment of *Lactobacillus acidophilus* into alginate beads for the effective treatment of cold restraint stress induced gastric ulcer. *Food Fun* 3 (1):83–90. doi: 10.1039/C1FO10100E.

Sun, M. C., P. P. Hou, X. Y. Wang, C. H. Zhao, B. J. Cheng, Y. L. Wang, H. W. Hao, T. H. Zhang, and H. Q. Ye. 2018. Pretreatment with *Lactobacillus reuteri* F-9-35 attenuates ethanol-induced gastric injury in rats. *Food. Nutr. Res* 62:62. doi: 10.29219/fnr.v62.1469.

Suo, H., X. Zhao, Y. Qian, P. Sun, K. Zhu, J. Li, and B. Sun. 2016. *Lactobacillus fermentum* Suo attenuates HCl/ethanol-induced gastric injury in mice through its antioxidant effects. *Nutr* 8 (3):155. doi: 10.3390/nu8030155.

Tarnawski, A., A. Ahluwalia, and M. K. Jones. 2013. Gastric cytoprotection beyond prostaglandins: Cellular and molecular mechanisms of gastroprotective and ulcer healing actions of antacids. *Curr. Pharm. Des* 19 (1):126–132. doi: 10.2174/138161213803903146.

Virchenko, O. V., T. M. Falalyeyeva, T. V. Beregova, M. Y. Spivak, L. M. Lazarenko, and O. M. Demchenko. 2015. Effects of mono-, poly-and composite probiotics on the ulceration caused by restraint stress. *Fiziolohichnyi Zhurnal (Kiev, Ukraine: 1994)* 61 (1):35–41. doi: 10.15407/fz61.01.035.

Wang, R., X. Zeng, B. Liu, R. Yi, X. Zhou, J. Mu, and X. Zhao. 2020a. Prophylactic effect of *Lactobacillus plantarum* KSFY06 on HCl/ethanol-induced gastric injury in mice. *Food. Func* 11 (3):2679–2692. doi: 10.1039/C9FO02474C.

Wang, R., K. Zhou, R. Xiong, Y. Yang, R. Yi, J. Hu, W. Liao, and X. Zhao. 2020b. Pretreatment with *Lactobacillus fermentum* XY18 relieves gastric injury induced by HCl/ethanol in mice via antioxidant and anti-inflammatory mechanisms. *Drug. Des. Dev. Ther* Volume 14:5721–5734. doi: 10.2147/DDDT.S280429.

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

Yang, R., X. Zhao, W. Wu, and J. Shi. 2021. Potential of probiotics for use as functional foods in patients with non-infectious gastric ulcer. *Trends Food Sci. Technol.* 111:463–474. doi: 10.1016/j.tifs.2021.02.070.

Yao, M., J. Xie, H. Du, D. J. McClements, H. Xiao, and L. Li. 2020. Progress in microencapsulation of probiotics: A review. *Compr. Rev. In Food Sci. Food Saf* 19 (2):857–874. doi: 10.1111/1541-4337.12532.

Yu, P., C. Ke, J. Guo, X. Zhang, and B. Li. 2020. *Lactobacillus plantarum* L15 alleviates colitis by inhibiting LPS-mediated NF-κB activation and ameliorates DSS-induced gut microbiota dysbiosis. *Front. Immunol* 11:575173. doi: 10.3389/fimmu.2020.575173.