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Pathology

Potential Biotherapeutic Effect of *Lactobacillus reuteri* NA1 on The Treatment of Induced Peptic Ulcer in Rats: Histopathological Study

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ABSTRACT

Peptic ulcer disease (PUD) is still a global public health problem. Development of novel therapies became necessary to overcome the limitations of traditional therapies. In this study we intend to treat experimentally induced peptic ulcer by using a new probiotic bacterial strain. *Lactobacillus reuteri* NA1 was isolated from peptic ulcer human-biopsy on MRS medium, molecularly identified, and registered in GenBank with accession number MH714546. This study was conducted on 2 experiments, the first experiment included 48 rats divided into 4 groups. Saline group, negative control group (saline); H.p. 1 group, H. pylori -ve stool administrated group; H.p. 2 group, H. pylori +ve stool administrated group; H.p. 3 group, H. pylori induced ulcer and treated with *L. reuteri* NA1. The second experiment included 36 rats divided into 3 groups. Saline group, negative control group (saline); As. 1 group, aspirin induced ulcer group; As. 2 group, aspirin induced ulcer and treated with *L. reuteri* NA1. Histopathological investigations showed a significant recovery of the experimentally induced gastric ulcers by both *H. pylori* and Aspirin after 6 weeks of the treatment with *L. reuteri NA1.* Complete healing of gastric mucosa was observed after 12 weeks of daily administration of *L. reuteri NA1*. *L. reuteri NA1* has shown a promising solution for treatment of peptic ulcer in *H. pylori*- antibiotic resistance patients, and aspirin users.

Keywords*: Peptic ulcer, Lactobacillus reuteri NA1, Probiotics, Helicobacter pylori, Histopathology.*

INTRODUCTION:

Peptic Ulcer Disease (PUD) therapy was mainly depending on stomach-acid-suppressing drugs to restore the balance between stomach acid secretion and mucosal integrity (Mössner, 2016). However, the ulcer recurrence rate is still high because stomach-acid-suppressing drugs cannot eliminate the true reasons of PUD (Arakawa *et.*, 2012)

The discovery of *Helicobacter pylori (H. pylori)* revolutionized the traditional strategies of PUD treatment and the antibiotic therapy became a part of PUD treatment plane, where *H. pylori* bacteria colonize the stomachs of more than half world population, and about 10-20% of *H. pylori* infected individuals develop peptic ulcers (Kuipers & Festen,1995). PUD notably increases between elder population due to increase the consumption of nonsteroidal

anti-inflammatory drugs (NSAIDs) such as ibuprofen and aspirin (Malfertheiner *et al.*, 2020).

Aspirin are the most described drugs in the routine clinical practice due their rapid effect in pain killing and anti-inflammation, moreover daily low aspirin dosage (less than 300mg or less 162 mg) is widely used especially between elders as anti-coagulant that protect from ischemic stroke and heart disease (Cleland, 2002). Aspirin play a direct role in induction of peptic ulcer via inhibition of stomach synthesis of prostaglandins (PGS) and expression of cyclooxygenase isoforms (COX- 1 and COX- 2). PGs and COX are necessary for mucosal integrity and spontaneous ulcer healing by enhancement of mucosal blood flow, secretion of mucous, and bicarbonate synthesis (Takeuchi & Amagase, 2018).

Generally, *H. pylori*-related antibiotic therapy and raising awareness of using aspirin between cardiologists and physicians, contributing to decrease the peptic ulcer prevalence which is globally estimated between 5-10% (Lanas &Chan, 2017). However, the mortality rate due PUD- related complications such as bleeding and proliferation has not significantly changed. Additionally, *H. pylori*- antibiotic resistance is globally raising which accompanied with sever antibiotic side effects therefore the need to modify the traditional drugs and finding novel therapies is a great Challenges (Malmi *et al.*, 2016).

Probiotics are non-pathogenic microorganisms found in different body sites wich play a vital role in maintain body health (Lebeer *et al.*, 2008). Numerous studies have proven that probiotics can be a promising solution for PUD treatment to avoid the side effects of PUD traditional therapies (Koder *et al.,* 2016)

Lactobcillus reuteri one of the few probiotic bacteria that has highly tolerant compatibility with the stomach where it can survive and live in very low pH of stomach environment, resist against bile salt secretion and colonize GIT (Krumbeck *et al.,* 2016).

L. reuteri is able to form biofilms (Salas-Jara *et al.,* 2016), attach to mucin and epithelial cell of GIT (Li *et al.,* 2008). Also *L. reuteri* can secrete mucus-binding proteins (MUBs) and MUB-like proteins, which are encoded by Lactobacillus specific clusters of orthologous protein coding genes (Gunning *et al.,* 2016). The secreted MUBs help *L. reuteri* to perfectly attachment to mucus layer of stomach and initiate the colonization (Roos & Jonsson, 2002). *L. reuteri* possess a profile of numerous metabolites with potential antimicrobial and immunomodulatory effect, such as vitamins, lactic acid, ethanol, acetic acid reutericyclin, and reuterin.

In *vitro* and in *vivo* studies beside clinical evidence have shown that, *L. reuteri* has a potential role in healing and/or preventing peptic ulcers which induced by different agents such as stress, smoking, acetic acid, ethanol, NSAIDs and *H. pylori.* In cell line studies, *L. reuteri* has a potential ability to inhabit binding of *H. pylori* to epithelial glycolipids therefore may prevent *H. pylori* infection in early stage (Mukai *et al.*, 2002). Animal studies have shown that different strains of *L. reuteri* have significant role in healing of peptic ulcer or its prevention. *L. reuteri F-9-35* has a potential gastro-protective activity**,** which improve mucus secretion and inhibited the oxidative stress and inflammatory response of gastric ulcer induced by ethanol in rats (Sun *et al.,* 2018).In clinical studies, a significant depression in ^{13}C-urea breath test and *H. pylori*-stool antigen test (HpSA) measurements was recorded after *L. reuteri ATCC 55730* supplementation for 28 days which indicates that *L. reuteri* is able to reduce *H. pylori* bacterial load in gastric mucosa and improve dyspeptic symptoms (Francavilla *et al.,* 2008). Also, numerous clinical trials have shown that, the significant role of *L. reuteri* to reduce the side effects accompanied to *H. pylori* – antibiotic therapy and raise the eradication rate (Lionetti *et al.,* 2006). The aim of this study was targeted to isolate a new strain of *L. reuteri* which can treat the peptic ulcer induced

chemically by aspirin and biologically by *H. pylori*.

MATERIALS AND METHODS

1. Isolation and identification of probiotic bacteria

1.1. Sample collection and transportation

Peptic ulcer biopsies were collected from *H. pylori*- positive volunteers in GI endoscope unit in Alexandria medical research institute, Egypt. Tissue biopsy was placed in sterilized normal saline and transported to microbiological lab within 2 hours for isolation of probiotic bacteria.

1.2.Culturing

Probiotic bacteria were isolated on DeMan-Rogosa- Sharpe (MRS- Oxoid) media according to perversely described methods (De Man *et al.*, 1960). After incubation, *lactobacillus* colonies were selected, a single colony from each plate was sub-cultured on MRS medium and incubated under anaerobic condition at 37ºC for 1 day. Repetition of a single colony sub-culturing 3 times were achieved to ensure getting pure bacterial strain plate.

1.3. Preservation

Single colony from pure strain MRS plate was cultured in 5 ml MRS broth tubes and statically incubated overnight under anaerobic conditions at 37 ºC. Fresh overnight broth cultures were centrifuged at 4000 rpm for 5 minutes, supernatant was removed and 500 µL 10% MRS- glycerol were added to the cell pellet and preserved frozen at -80ºC (Howard, 1956)

1.4. Microbiological examination

According to gram staining protocol a single colony from fresh pure plate and 10 µl isolated bacterial broth were gram- stained and the cells were examined by light microscope (Coico, 2006).

1.5. Molecular identification

The 16S rRNA gene was amplified (Biometra thermocycler, Germany) and sequenced (Applied Biosystems® 3500 Genetic Analyzers) using primers; Bact 27f (5′-AGA GTT TGATC(A/C)-TGG CTCAG-3), Bact1492r (5-TACGG (C/T)-ACC TTG TTA CGACTT-3) and Bact 1098r (5-AAG GGT TGC GCT CGT TGCG- 3) (Chang et al. 2000; Picard et al. 1999). The obtained 16S rRNA sequences of the new isolate were aligned with similar sequences on genebank using BLAST searching (http://www.ncbi.nlm.nih.gov/blast/), the nearest similar sequences were downloaded in Fasta format programmer and aligned using Clustal W implemented in MEGA software version 3,1.

2. Experimental design

Ethical approval: Genetic Engineering and Biotechnology Research institute (GEBRI), university of Sadat city, Committee for animal Research Ethics reviewed and formally approved this study.

Animal model: Experiments were achieved on healthy female Wistar rats. Rats were obtained and experimented at Animal House of Genetic Engineering and Biotechnology Research institute (GEBRI), university of Sadat city-Egypt. Rats fed on standard diet pellets and tap water.

2.1. **First experiment**

First experimental design is explained in next table (1).

Groups	**Description**	**Rats number**	**Zero day**	**1st check point (25 week)**	**Treatment with *L. reuteri NA1* (26^{th} – *37 week)***	**2nd check point (31 week)**	**3rd check point (37 week)**
Saline group	Negative control group (saline).	12 rats in each group weighted 50-70g	Saline	HpSA tests in rat feces Urease activity in stomach tissues Microbiological detection of H. pylori in stomach tissues Histopathology for stomach tissues	-	HpSA tests in rat feces Histopathology for stomach tissues	HpSA tests in rat feces Histopathology for stomach tissues
H.p. 1	*H. pylori* -ve stool administrated group		Processed *H. pylori* -ve stool		-		
H.p. 2	*H. pylori* +ve stool administrated group		Induction of H. pylori infection on rats via processed *H. pylori* +ve human feces		+		
H.p. 3	*H. pylori* induced ulcer and treated with *L. reuteri NA1*				+		

Table (1): shows rat groups, time plan and methodology of the first experiment.

2.1.1. Induction of *H. pylori* infection on rats via human feces

Fresh feces samples were provided by a volunteer man 42 years old suffered from intensive infection of *H. pylori*, patient was not taking any drugs for *H. pylori* treatment before or during this study. These samples were processed according to (*Kelly et al.,* 1994) and the obtained bacterial deposits was dissolved in normal saline to which *H. pylori*-selective antibiotics were added (Siu *et al.,* 1998)

2.1.2. Rat preparation and feeding with processed human feces

Rats were fasted for 7 hours, then the stomach were alkalized by oral administration of 200 µl of 0.2 M NaHCO3 to neutralize gastric acidity of fasted rat. After 15 min of alkalization rats were received the first fresh *H. pylori* positive processed feces to H.p. 2 and H.p. 3 groups (2 ml for each rat). Then rats were fasted again another 7 hours, then the same dose were given again. Rats after the second dose by one hour can freely feed normally. Fresh infectious doses were daily administrated for 25 weeks. Rats in group H.p 1 subjected to the same protocol but with *H. pylori*- negative stool samples, while rates in saline group were received 2 mL saline by gastric gavage (Werawatganon, 2014).

2.1.3. Detection of *H. pylori* infection, 1st check point (at week 25):

In rat feces

After 25 weeks of administrating *H. pylori* infectious doses, rectal stool samples of each rat were collected by abdominal pressure and *rapid H. pylori* stool antigen (HpSA) tests were achieved according to the manufacturer's instructions (SD Bioline *H. pylori* Ag kit, Standard Diagnostics, Inc) (Moon *et al.,* 2013).

In stomach tissues

After 25 weeks of administrating *H. pylori* infectious doses, 4 rats were selected randomly from each group to conduct the following tests. After 20 hours fasting, rats were anaesthetized with ether and their stomachs were collected. Each stomach was opened and washed by PBS (PH7), then about 4x4 mm portions of each stomach was placed directly in 500 µL sterile urea broth (Oxoid) containing phenol red

indicator tube to examine the urease activity (Uotani & Graham, 2015). Another 4x4 mm portions of each stomach was placed in cryovial containing 500 µL BHI-VAN broth (Siu *et al.*, 1998), transported in an ice box (about 4ºC) to the microbiological laboratory within 2 hours for culturing and microbiological examination for detection of *H. pylori* in stomach tissues (Han *et al.*, 1995). Stomach samples from all groups were collected and placed in 10 % neutral buffered formalin for histopathological examination.

2.14. Preparation of *L. reuteri NA1* treatment doses:

Overnight *L. reuteri NA1* was grown on MRS broth until log phase, centrifuged at 6,000 rpm for 10 minutes, supernatant was removed, cell pellet was washed twice with sterile PBS (PH 7) and re-suspended in normal saline for direct intra- gastric gavaging in rats of H.p 3 group (Sun *et al.*, 2018) .

2.1.5. Rat preparation and administration of *L. reuteri NA1* treatment doses:

After induction of peptic ulcer rats of H.p. 3 group were fasted for 10 hours and each rat was received an oral dose of fresh prepared *L. reuteri NA1* (10^8 CFU/ gram body weight/ day) for 12 weeks. Rats of other groups were received normal saline with a dose of 2 mL.

2.1.6. Evaluation the Biotherapeutic effect of *L. reuteri NA1*, 2nd check point (at week 31) and 3rd check point (at week 37).

At week 31 and week 37 of the experiment respectively, 4 rats were selected randomly from each group to conduct the following tests:

H. pylori eradication

For checking the *H. pylori* eradication, HpSA tests were applied on *H. pylori* infected rats (H.p. 3 group) after 6 and 12 weeks of daily administration of *L. reuteri NAI* treatment doses.

Histopathological examination

After 72 hours fixation in 10 % neutral buffered formalin (pH 7.4), biopsies were washed, dehydrated, embedded in paraffin wax, serially sectioned with a microtome at 3 µm thickness and stained with hematoxylin and eosin for histopathological investigations. Leica DMLB microscopes were used in this study and histological photos were taken by using Leica Camera.

2.2. **Second experiment**

Second experimental design is explained in next table (2)

Groups	**Description**	**Rat number**	**Zero day**	**1st check point (2nd day)**	**Treatment with *L. reuteri NA1 (2nd – 14th week)***	**2nd check point (7 week)**	**3rd check point (13th week)**
Saline group	Negative control group (saline).	12 rats in each group weighted 120-150g	Saline 2 ml	Histopathology for stomach tissues	-	Histopathology for stomach tissues	Histopathology for stomach tissues
As.1 group	Aspirin induced ulcer group		Aspirin 500 mg/kg BW		-		
As.2 group	Aspirin induced ulcer and treated with *L. reuteri NA1*		Aspirin 500 mg/kg BW		+		

Table (2): shows rat groups, time plan and methodology of the first experiment.

2.2.1. Induction of gastric ulcer via aspirin

Aspirin tablets (Rivo® tablets, each containing 320 mg acetyl salicylic acid) was purchased from the Arab Drug Company (ADCO) for Pharmaceuticals and Chemical Industries (Cairo, Egypt). Tablets were grinded, weighted and suspended in warm water. Each rat in As.1 group and As.2 group was daily fasted for 24 hours then received a single oral dose of hydrolyzed Aspirin at a dose of 500 mg/kg body weight/ day (Shah & Patel, 2012).

2.2.2. Detection of aspirin induced peptic ulcer, 1st check point (at 2nd day)

4 rats were randomly selected from each and subjected to histopathological investigation as described above.

2.2.3. Rat preparation and administration of *L. reuteri NA1* treatment doses

Rats and *L. reuteri NA1* treatment doses were prepared as described above in the first experiment. Rats in As.2 group were administrated *L. reuteri NA1* treatment doses and other groups given saline.

2.2.4. Evaluation the Biotherapeutic effect of *L. reuteri NA1*, 2nd check point (at week 7) and 3rd check point (at week13).

At the 7th week and the 13th week of the experiment, 4 rats were selected randomly from each group and subjected to histopathological investigation as described above.

RESULTS

1. Characterization of Isolated probiotic bacterial strain

Microbiologically: gram positive bacilli with long chain bacterial cells were detected by light microscope.

Molecularly: blast alignment of 16s rRNA gene resulted in a new strain of *L. reuteri NA1* which was registered on genbank under accession number MH714546**.**

2. First experiment

Results of the first experiment are summarized in table (3).

2.1. Evaluation of *H. pylori* infection and biotherapeutic effect of *L. reuteri NA1* in rats:

Rapid H. pylori Stool Antigene (HPSA) Test

after 25 weeks of administration of H. pylori infectious doses, infected rats of groups H.p.2 and H.p. 3 showed positive results by appearing red tested line of HpSA strips, and the test was still positive after 12 weeks of administration of L. reuteri NA1 tratment doses on H.p. 3 group, while control groups saline and H.p. 1 showed negative result by using HpSA strips.

Urease activity

after 25 weeks of administration of H. pylori infectious doses all embedded gastric biopsies in urease media tubes of H.p. 3 and H.p. 4 rats showed rapid positive urease activity (detected by changing the color of urea broth from yellow to red color) within less than 1 hour incubation at 37ºC under micro- aerobic condition, while biopsies of saline and H.p. 3 groups showed negative urease activity even after 24 hours incubation.

H.pylori culture and gram staining

After 25 weeks of administration of H. pylori infectious doses, cultured gastric biopsies of H.p. 3 and H.p. 4 rat groups showed H.pylori-positive cultures after 4- 5 days incubation in micro -aerobic conditions; tiny translucent colonies were appeared on H. pylori selective plates among contamination growth and gram-stained colonies showed curved shape of H. pylori cells. Saline and H.p.1 rat groups showed H.pylori negative cultures (3 plates were discarded due to contamination overgrowth).

Histological examination of gastric mucosa

Peptic ulcer was observed in gastric mucosa of rats in H.p. 2 group (H. pylori infected group after 25 weeks) (Figures 2 A & B). Partial regeneration of H. pylori induced peptic ulcer was observed after 6 weeks of administration of L. reuteri NA1 treatments does (Figures 2 C & D), while complete regeneration was recorded after 12 weeks of treatment (Figures 2 E & F). Gastric mucosa of in both saline and, H. pylori -ve stool (H.p. 1 group) administrated groups showed normal histological architectures (Figures 1 A & B).

Groups	**1st check point (25 week)**				**2nd check point (31 week)**		**3nd check point (37 week)**	
	HpSA test of rat stool	Urease activity stomach tissue	*H. pylori* culture of stomach tissue	Histopathology for stomach tissue	HpSA test of rat stool	Histopathology for stomach tissue	HpSA test of rat stool	Histopathology for stomach tissue
Saline group	-	-	-	Normal histoarchitectures	-	Normal histoarchitectures	-	Normal histoarchitectures
H.p. 1	-	-	-	Normal histoarchitectures	-	Normal histoarchitectures	-	Normal histoarchitectures
H.p. 2	+	+	+	Mucosal ulcer	+	Mucosal ulcer	+	Mucosal ulcer
H.p. 3	+	+	+	Mucosal ulcer	+	Partial mucosal regeneration	+	complete mucosal regeneration

Table (3): shows the HpAS, culture, and histopathologial results of the first experiment at the different check points.

2. Second experiment

Results of the first experiment are summarized in table (4).

2.1. Histopathological evaluation of induced stomach ulcers by aspirin

All rats of As.1 and As.2 groups developed sever gastric ulcers after administration of aspirin dose (figure 3), while control saline group C showed normal stomach integrity (figure 1).

2.2. Histopathological evaluation biotherapeutic effect of *L. reuteri NA1* in rats

Erosion of gastric mucosa was recorded after one dose of aspirin administration in rats of As. 1 group (Figures 3 A & B). Partial regeneration of aspirin induced mucosal erosions was observed after 6 weeks of administration of L. reuteri NA1 treatments does (Figures 3 C & D), while complete regeneration was recorded after 12 weeks of treatment (Figures 3 E & F).

Groups	**1st check point (2nd day)**	**2nd check point (7 week)**	**3rd check point (13th week)**
Saline group	Normal histoarchitectures	Normal histoarchitectures	Normal histoarchitectures
As.1 group	Mucosal erosion	Mucosal erosion	Mucosal erosion
As.2 group	Mucosal erosion	Partial mucosal regeneration	Complete mucosal regeneration

Table (4): shows histopathologial results of the second experiment at the different check points.

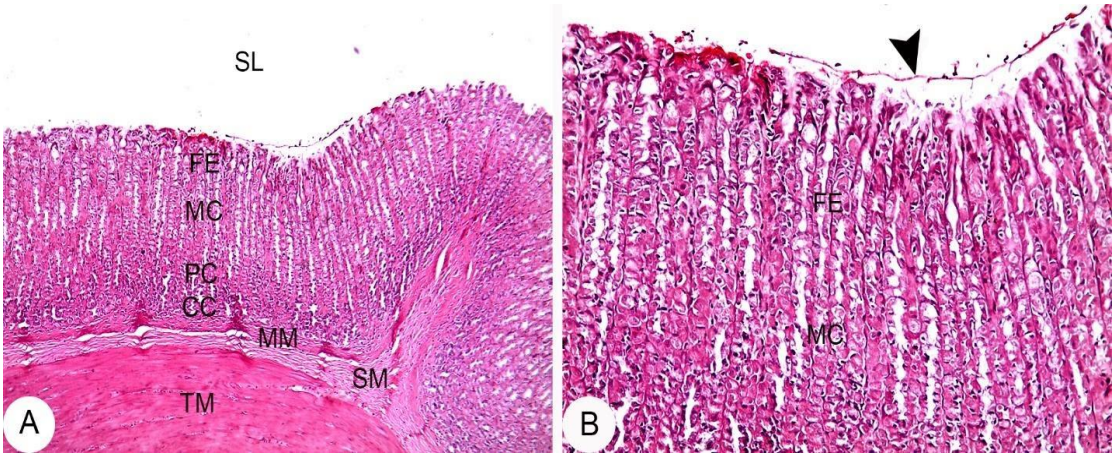

Figure (1): Normal fundic (glandular) epithelium, stomach, Wistar rat (rats of saline groups and H.p.1 group). **Sections A and B:** a foveolar epithelium (FE), mucous neck cells (MC), parietal cells (PC), chief cells (CC), lamina muscularis mucosa (MM), submucosa (SM) and tunica muscularis (TM), Stomach lumen (SL), mucosal surface (arrowhead). H&E, **A** X4, **B** X10.

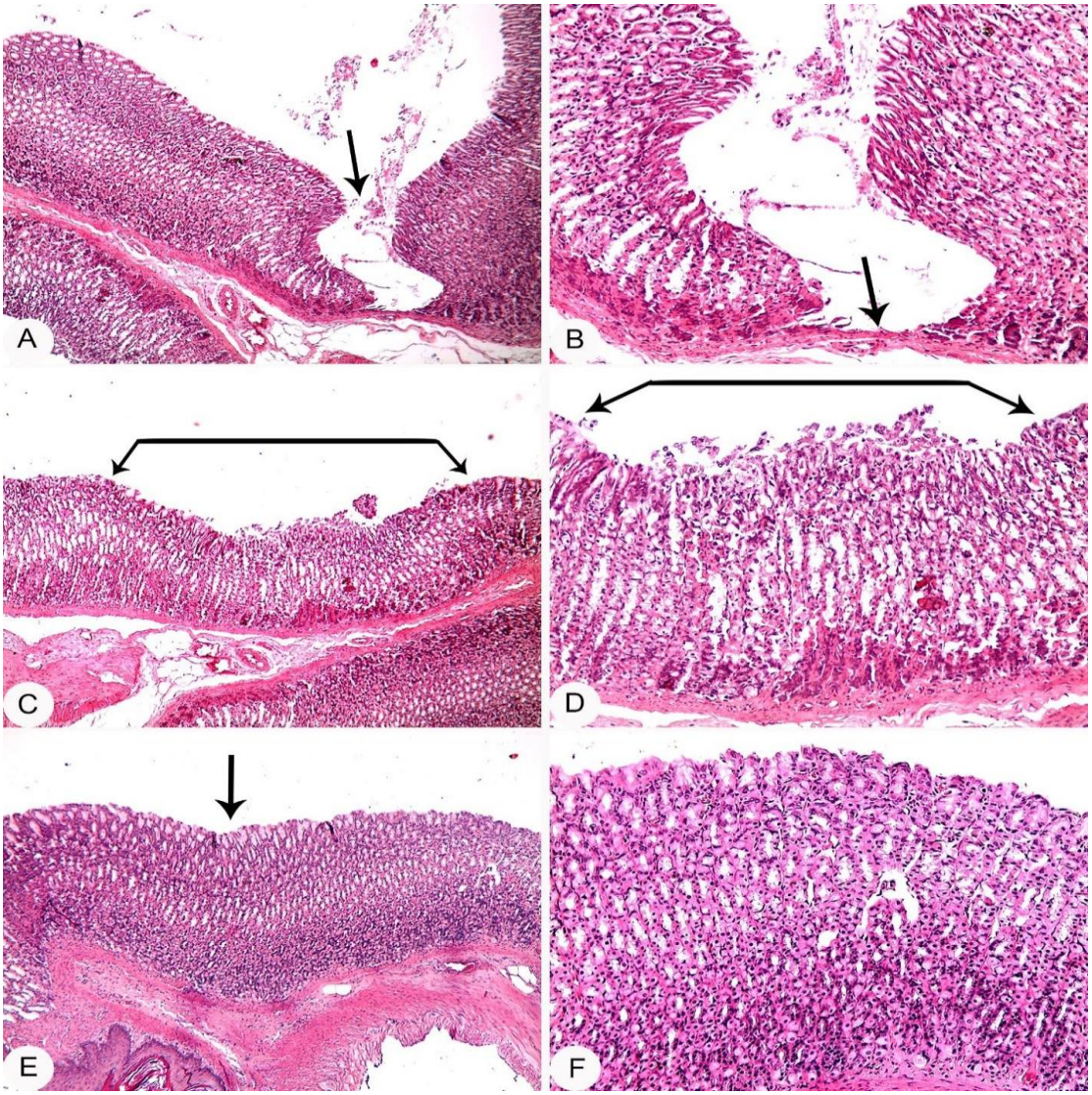

Figure (2): Fundic (glandular) epithelium, stomach, Wistar rats, *H. pylori* infected groups. **A & B)** H.p. 2 group; *H. pylori* infected group showed peptic ulcer in gastric mucosa (arrows). **C & D)** H.p. 3 group; after administration of *L. reuteri NA1* treatment doses for 6 weeks showed partial regeneration of H. pylori induced ulcer (marked areas). **E & F)** H.p. 3 group; after administration of *L. reuteri NA1* treatment doses for 12 weeks, indicated complete regeneration of *H. pylori* induced ulcer (arrow). H&E, **A, C, E** X4; **B, D, F** X10.

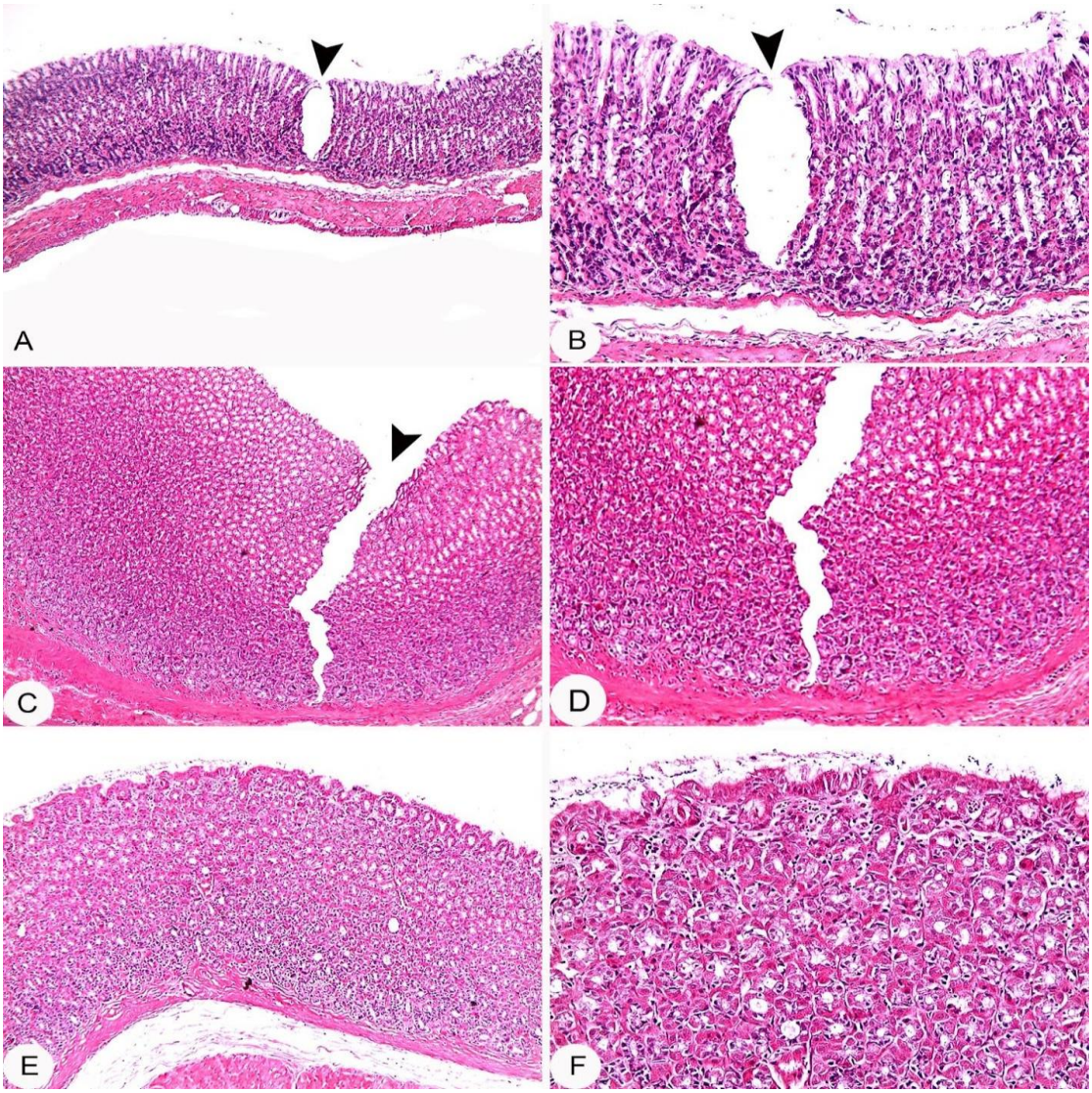

Figure (3): Fundic (glandular) epithelium, stomach, Wistar rat, aspirin treated groups. A & B) As. 1 group; showed erosion of gastric mucosa induced by aspirin (arrowheads). **C & D)** As. 2 group; after administration of *L. reuteri NA1* treatment doses for 6 weeks showed partial regeneration of the mucosal erosion (arrowhead). **E & F)** As. 3 group; after administration of *L. reuteri NA1* treatment doses for 12 weeks showed complete regeneration of gastric mucosa. H&E, **A, C, E** X4; **B, D, F** X10.

DISCUSSION

Evidence have proven that probiotics biotherapy can be a powerful, safe, cheap, and promising alternative therapy for many public health problems, such as infectious diseases, antibiotic- resistance, malnutrition, PUD and cancer, in particularly for developing countries where low economic resources (Nahaisi & RS, 2014).

Biotherapeutic ability is highly varying among different probiotic strains, and studies which evaluate the effects of probiotics on gastric ulcers are relatively limited (Khoder *et al.*, 2016); This may be related to aggressive physiological conditions in the stomach (such as very low PH, bile acids, and digestive enzymes) which make the survival and growth ability of probiotic strains is difficult. Therefore, in this study we appointed to isolate a new probiotic strain from peptic ulcer biopsies so the isolated probiotic strain may be highly adaptive to stomach environment.

H. pylori and NSAIDs/ Aspirin are classified as the most causative agents of PUD over worldwide (Kurata & Nogawa, 1997). *H. pylori* is one of the most causative factors of peptic ulceration over worldwide, and studies have shown that the clearance of *H. pylori* in infected asymptomatic patients is necessary to avoid the occurrence of peptic ulcers and gastric cancer in the future (Malfertheiner *et al.*, 2009).

Using *H .pylori* cultures to induce the *H. pylori* infection in rats is more controlled,

however Isolation of *H. pylori* from gastric biopsy samples is complicated and not always successful (Kusters *et al.,* 2006). In the present study we induced peptic ulcers by *H. pylori* infection from human feces which is economic, rapid and can be achieved in future studies on *H. pylori*-animal models.

H. pylori infection can be induced in mice models during a few days (Werawatganon, 2014), however arising of gastric lesions need long- term infection stats from 16 weeks and may be excess 80 weeks (Kim *et al.,* 2003). In the present study, histological examination of the gastric mucosa showed that, all rats of H.p. infected groups developed erosive and ulcerative lesions after 25 weeks of *H. pylori* infection.

Antibiotics is the approved therapy to eradicate *H. pylori* infection, however the need to alternative or complementary therapies (e.g. probiotics) became necessity. Antibiotic therapy has many risks such as failure of eradication of *H. pylori* infection due to development of antibiotic resistant- strains, as well as antibiotic- side effects (such as diarrhea, nausea or vomiting), breakdown of gut microbiome barriers and increase the severity of PUD (Kim *et al.,* 2015).

In *vitro* studies showed a potent anti- *H. pylori* effect of probiotics (Fijan, 2016). Conversely, In *vivo* and clinical trials showed limited role of probiotics in full eradication of *H. pylori* when probiotic were used instead of antibiotic therapy. Meta-analysis of previous studies with 403 cases has shown the lack of using probiotic strains as monotherapy in eradication of *H. pylori* (Losurdo *et al.,* 2018).

In this study, our results are compatible with the previous observations, where *H. pylori* diagnostic tests still positive after 12 weeks of administration of *L. reuteri NA1* treatment doses (table 3), which indicates the limited role of *L. reuteri NA1* in eradication of *H. pylori* infection. In contrast, using probiotic/ lactobacillus as supplementation with antibiotics has shown significant improvement in *H. pylori* eradication rate in addition to reducing the severe side effects of antibiotics (Lionetti *et al.,* 2006). Additionally, probiotic supplementation can restore the balance of gut microbiota (Ianiro *et al.,* 2014).

Meta- analysis included 45 articles with 6997 cases was conducted by (Zhang *et al.,* 2015) showed probiotics supplementation raised the success of antibiotics -standard triple therapy to eradicate *H. pylori* infection by 13% and decreased the antibiotic- side effects by ~ 41%. In Egypt, clinical study was achieved on *H. pylori* dyspeptic patients. *L. reuteri* supplementation along- side with triple therapy showed a fairly increasing in eradication rate by 8.6 %, however showed a significant improving in gastrointestinal symptom rating scale (GSRS), histological features, and reducing antibiotic side effects (Emara *et al.,* 2014).

In this study, *L. reuteri NA1* was not tested with antibiotics, and failed to *eradicate H. pylori* infection after the treatment duration, however *L. reuteri NA1* administration showed a notable improvement in the regeneration of gastric mucosa ulcer after 6th weeks (figure 2) and complete regeneration after 12 weeks (figure 2). Therefore, using of *L. reuteri NA1* as monotherapy may be purposive in case of *H. pylori*- antibiotic resistant patients and *asymptomatic H. pylori* infected patients where antibiotic therapy is not preferred (Malfertheiner *et al.,* 2002). Using of *L. reuteri NA1* may be an alternative therapy for healing or preventing PUD, and alternative for high costly antibiotic therapy particularly for developing countries.

In general, as in western health guidelines, public health systems in developing countries should be reviewed. The co-prescription of probiotics with antibiotics in routine clinical practices should be described to decrease disruption of gut microbiota and reduce the antibiotic side effects (Hojsak *et al.,* 2018).

Investigations have proven that probiotics are not only effective against gastric ulcer induced by stress or *H. pylori*, but also play major role in treatment or prevention of ulcers induced by chemical agents and drugs such as ethanol, acetic acid and by NSAIDs/ aspirin. Other

lactobacillus strains such as *L. reuteri DSM 17938* significantly reduced the ulcer area induced in rats by 50% ethanol. Anther mutant *L. reuteri F-9-35* reduced inflammatory response by improving mucus secretion, biosynthesis of glutathione and nitric oxide, also down-regulating gene expression of cyclooxygenase /COX-2 mRNA of injured gastric tissue induced by ethanol in rats (Sun *et al.*, 2018)

In this study, a single oral dose of aspirin (500 mg/kg body weight) induce a direct symptomatic gastric mucosal injury in rats. Histopathologically, 6 weeks administration of *L .reuteri NA1* treatment doses showed remarkable recovery gastric ulcers by regeneration and increasing the thickness of the mucosal layer (figure 3), while complete ulcer healing was recorded after 12 weeks of *L .reuteri NA1* treatment doses (figure 3). These results indicate *L. reuteri NA1* supplementation may be helpful to the regeneration of destructed gastric mucosa in aspirin users.

From the previous studies and our results, we suppose that *L. reuteri* probiotic bacteria administration can help in reducing gastric mucosal erosion and ulceration by several mechanisms such as, reduction of pathogenic bacterial load and/or producing agents which can help directly or indirectly in the healing process, but these hypotheses need further studies for confirmation.

CONCULSION

Finally, *L. reuteri NA1* is a new strain isolated from human origin and showed potent bio-therapeutic effect against peptic ulcer induced in rats by the two most ulcer-causative agents, *H. pylori* and Aspirin. The histopathological results indicated a complete recovery of gastric ulcer lesions after 12 weeks of applying *L. reuteri NA1* treatment doses in rats. *L. reuteri NA1* may be a promising anti-ulcer biotherapy in cases of PUD, *H. pylori* antibiotic resistant patients and aspirin users.

REFRENCES

Arakawa, T., Watanabe, T., Tanigawa, T., Tominaga, K., Fujiwara, Y., & Morimoto, K. I. (2012). Quality of ulcer healing in gastrointestinal tract: its pathophysiology and clinical relevance. *World Journal of Gastroenterology: WJG*, *18*(35), 4811.

Chang, Y. J., Stephen, J. R., Richter, A. P., Venosa, A. D., Brüggemann, J., Macnaughton, S. J., ... & White, D. C. (2000). Phylogenetic analysis of aerobic freshwater and marine enrichment cultures efficient in hydrocarbon degradation: effect of profiling method. *Journal of Microbiological Methods*, *40*(1), 19-31.

Cleland, J. G. (2002). Preventing atherosclerotic events with aspirin. *Bmj*, *324*(7329), 103-105.

Coico, R. (2006). Gram staining. *Current protocols in microbiology*, (1), A-3C.

De Man, J. C., Rogosa, M., & Sharpe, M. E. (1960). Cultivation medium for *lactobacilli*. *J. Appl. Bacteriol*, *23*, 130-135.

Emara, M. H., Mohamed, S. Y., & Abdel-Aziz, H. R. (2014). Lactobacillus reuteri in management of *Helicobacter pylori* infection in dyspeptic patients: a double-blind placebo-controlled randomized clinical trial. *Therapeutic Advances in Gastroenterology*, 4–13.

Fijan, S. (2016). Antimicrobial effect of probiotics against common pathogens. *Probiotics and prebiotics in human nutrition and health*, 191-221.

Francavilla, R., Lionetti, E., Castellaneta, S. P., Magistà, A. M., Maurogiovanni, G., Bucci, N., ... & Miniello, V. L. (2008). Inhibition of *Helicobacter pylori* infection in humans by Lactobacillus reuteri ATCC 55730 and effect on eradication therapy: a pilot study. *Helicobacter*, *13*(2), 127-134.

Gunning, A. P., Kavanaugh, D., Thursby, E., Etzold, S., MacKenzie, D. A., & Juge, N. (2016). Use of atomic force microscopy to study the multi-modular interaction of bacterial adhesins to mucins. *International journal of molecular sciences*, *17*(11), 1854.

Han, S. W., Flamm, R., Hachem, C. Y., Kim, H. Y., Clarridge, J. E., Evans, D. G., ... & Graham, D. Y. (1995). Transport and storage of *Helicobacter pylori* from gastric mucosal biopsies and clinical isolates. *European Journal of Clinical Microbiology and Infectious Diseases*, *14*(4), 349-352.

Hojsak, I., Fabiano, V., Pop, T. L., Goulet, O., Zuccotti, G. V., Çokuğraş, F. C., ... & Kolaček, S. (2018). Guidance on the use of probiotics in clinical practice in children with selected clinical conditions and in specific vulnerable groups. *Acta Paediatrica*, *107*(6), 927-937.

Howard, D. H. (1956). The preservation of bacteria by freezing in glycerol broth. *Journal of bacteriology*, *71*(5), 625-625.

Ianiro, G., Bibbò, S., Gasbarrini, A., & Cammarota, G. (2014). Therapeutic modulation of gut microbiota: current clinical applications and future perspectives. *Current drug targets*, *15*(8), 762-770.

Kelly, S. M., Pitcher, M. C., Farmery, S. M., & Gibson, G. R. (1994). Isolation of *Helicobacter pylori* from feces of patients with dyspepsia in the United Kingdom. *Gastroenterology*, *107*(6), 1671-1674.

Khoder, G., Al-Menhali, A. A., Al-Yassir, F., & Karam, S. M. (2016). Potential role of probiotics in the management of gastric ulcer. *Experimental and therapeutic medicine*, *12*(1), 3-17.

Kim, D. H., Kim, S. W., Song, Y. J., Oh, T. Y., Han, S. U., Kim, Y. B., ... & Hahm, K. B. (2003). Long-term evaluation of mice model infected with *Helicobacter pylori*: focus on gastric pathology including gastric cancer. *Alimentary pharmacology & therapeutics*, *18*, 14-23.

Kim, S. Y., Choi, D. J., & Chung, J. W. (2015). Antibiotic treatment for *Helicobacter pylori*: Is the end coming?. *World journal of gastrointestinal pharmacology and therapeutics*, *6*(4), 183.

Krumbeck, J. A., Marsteller, N. L., Frese, S. A., Peterson, D. A., Ramer-Tait, A. E., Hutkins, R. W., & Walter, J. (2016). Characterization of the ecological role of genes mediating acid resistance in *Lactobacillus reuteri* during colonization of the gastrointestinal tract. *Environmental microbiology*, *18*(7), 2172-2184.

Kuipers, E. J., Thijs, J. C., & Festen, H. P. (1995). The prevalence of *Helicobacter pylori* in peptic ulcer disease. *Alimentary pharmacology & therapeutics*, *9*, 59-69.

Kurata, J. H., & Nogawa, A. N. (1997). Meta-analysis of risk factors for peptic ulcer: nonsteroidal antiinflammatory drugs, *Helicobacter pylori*, and smoking. *Journal of clinical gastroenterology*, *24*(1), 2-17.

Kusters, J. G., Van Vliet, A. H., & Kuipers, E. J. (2006). Pathogenesis of *Helicobacter pylori* infection. *Clinical microbiology reviews*, *19*(3), 449-490.

Lanas, A., & Chan, F. K. (2017). Peptic ulcer disease. *The Lancet*, *390*(10094), 613-624.

Lebeer, S., Vanderleyden, J., & De Keersmaecker, S. C. (2008). Genes and molecules of *lactobacilli* supporting probiotic action. *Microbiology and Molecular Biology Reviews*, *72*(4), 728-764.

Li, X. J., Yue, L. Y., Guan, X. F., & Qiao, S. Y. (2008). The adhesion of putative probiotic lactobacilli to cultured epithelial cells and porcine intestinal mucus. *Journal of Applied microbiology*, *104*(4), 1082-1091.

Lionetti, E., Miniello, V. L., Castellaneta, S. P., Magista, A. M., De Canio, A., Maurogiovanni, G., ... & Francavilla, R. (2006). Lactobacillus reuteri therapy to reduce side-effects during anti-*Helicobacter pylori* treatment in children: a randomized placebo controlled trial. *Alimentary pharmacology & therapeutics*, *24*(10), 1461-1468.

Losurdo, G., Cubisino, R., Barone, M., Principi, M., Leandro, G., Ierardi, E., & Di Leo, A. (2018). Probiotic monotherapy and *Helicobacter pylori* eradication: a systematic review with pooled-data analysis. *World journal of gastroenterology*, *24*(1), 139.

Malfertheiner, P., & Schulz, C. (2020). Peptic Ulcer: Chapter Closed?. *Digestive Diseases*, *38*(2), 112-116.

Malfertheiner, P., Chan, F. K., & McColl, K. E. (2009). Peptic ulcer disease. *The lancet*, *374*(9699), 1449-1461.

Malfertheiner, P., Megraud, F., O'morain, C., Hungin, A. P. S., Jones, R., Axon, A., ... & European Helicobacter Pylori Study Group (EHPSG). (2002). Current concepts in the management of *Helicobacter pylori infection*—The Maastricht 2-2000 Consensus Report. *Alimentary pharmacology & therapeutics*, *16*(2), 167-180.

Malmi, H., Kautiainen, H., Virta, L. J., & Färkkilä, M. A. (2016). Increased short-and long-term mortality in 8146 hospitalised peptic ulcer patients. *Alimentary pharmacology & therapeutics*, *44*(3), 234-245.

Moon, D. I., Shin, E. H., Oh, H. G., Oh, J. S., Hong, S., Chung, Y., & Kim, O. (2013). Usefulness of a *Helicobacter pylori* stool antigen test for diagnosing H. pylori infected C57BL/6 mice. *Laboratory animal research*, *29*(1), 27-32.

Mössner, J. (2016). The indications, applications, and risks of proton pump inhibitors: A review after 25 years. *Deutsches Ärzteblatt International*, *113*(27-28), 477.

Mukai, T., Asasaka, T., Sato, E., Mori, K., Matsumoto, M., & Ohori, H. (2002). Inhibition of binding of *Helicobacter pylori* to the glycolipid receptors by probiotic Lactobacillus reuteri. *FEMS Immunology & Medical Microbiology*, *32*(2), 105-110.

Nahaisi, M. H., & RS, N. G. (2014). Probiotics as a strategy to Improve overall human health in developing countries. *J Prob Health*, *2*(118).

Picard FJ, Martineau F, Me´nard C, Roy PH, Onellette M, Bergeron MG (1999) Development of a PCR assay for rapid detection of*Enterococci*. J Clin Microbiol 37:3497–3503.

Roos, S., & Jonsson, H. (2002). A high-molecular-mass cell-surface protein from *Lactobacillus reuteri 1063* adheres to mucus componentsThe GenBank accession number for the sequence reported in this paper is AF120104. *Microbiology*, *148*(2), 433-442.

Salas-Jara, M. J., Ilabaca, A., Vega, M., & García, A. (2016). Biofilm forming Lactobacillus: new challenges for the development of probiotics. *Microorganisms*, *4*(3), 35.

Shah, J. S., & Patel, J. R. (2012). Anti-ulcer activity of Lucer against experimentally induced gastric ulcers in rats. *Ayu*, *33*(2), 314.

Siu, L. K., Leung, W. K., Cheng, A. F. B., Sung, J. Y., Ling, T. K. W., Ling, J. M. L., & Chung, S. C. S. (1998). Evaluation of a selective transport medium for gastric biopsy specimens to be cultured for *Helicobacter pylori*. *Journal of clinical microbiology*, *36*(10), 3048-3050.

Sun, M. C., Hou, P. P., Wang, X. Y., Zhao, C. H., Cheng, B. J., Wang, Y. L., ... & Ye, H. Q. (2018). Pretreatment with *Lactobacillus reuteri F-9-35* attenuates ethanol-induced gastric injury in rats. *Food & nutrition research*, *62*.

Takeuchi, K., & Amagase, K. (2018). Roles of cyclooxygenase, prostaglandin E2 and EP receptors in mucosal protection and ulcer healing in the gastrointestinal tract. *Current pharmaceutical design*, *24*(18), 2002-2011.

Uotani, T., & Graham, D. Y. (2015). Diagnosis of Helicobacter pylori using the rapid urease test. *Annals of translational medicine*, *3*(1).

Werawatganon, D. (2014). Simple animal model of Helicobacter pylori infection. *World Journal of Gastroenterology: WJG*, *20*(21), 6420.

Zhang, M. M., Qian, W., Qin, Y. Y., He, J., & Zhou, Y. H. (2015). Probiotics in Helicobacter pylori eradication therapy: a systematic review and meta-analysis. *World Journal of Gastroenterology: WJG*, *21*(14), 4345.