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ORIGINAL ARTICLE

The Healing Effect of Probiotics on Indomethacin Induced Gastric Ulcer in Rats

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ABSTRACT

Background*: Probiotics are living organisms which upon ingestion in certain numbers exert health benefits beyond inherent basic nutrition, they are used for the treatment of Gastric ulcer. The lactobacillus rhamnosus, Bifidobacterium bifidum, Lactococcus lactis and Saccharomyces cerevisiae are the most significant groups of probiotic organisms.* ***Objectives****: The aim of this study is to investigate the effect of a probiotic strains for treatment of peptic ulcer.* ***Methodology:*** *Gastric ulcers were induced by indomethacin in rats .probiotics were given for three days. The strains significantly reduced gastric ulcer area as compared to control and negative control groups. Neutrophils were strongly increased for treatment of peptic ulcer.* ***Results:*** *The results showed that lactobacillus rhamnosus, Bifidobacteria, Lactococcus lactis and Saccharomyces cerevisiae that used in dairy milk products and yogurt enhanced gastric ulcer healing via stimulating immune system.*

INRODUCTION

Gastric ulceration is a benign lesion on the mucosal epithelium upon exposure of the stomach to excess acid and aggressive pepsin[1]. It is the most prevalent gastrointestinal disorder ever known, accounting for an estimated 15 mortality out of every 15,000 complications yearly in the world.[2] Various synthetic antiulcer drugs are presently available and some of these like cimetidine, misoprostol, ranitidine, omeprazole and esomeprazole are employed to manage and cure NSAID-induced gastric ulcer. However, each of these drugs confers simpler to severe side effects, prompting a search for non-toxic, easily accessible and affordable antiulcer medication [3].

Currently, the idea of food with microorganisms purposely added to the food seems startling since there is an emphasis on omitting microorganisms that cause food-borne illness from food. There is a growing interest in the consumption of probiotic foods due to their reported health benefits. Probiotics are defined as live microbial food ingredients that have a beneficial effect on human health [4].

Probiotics have a long history. The Old Testament suggested that Abraham lived along and healthy because of consuming sour milk which has living microorganisms. In 20th century Metchnikoff suggested that the long, healthy life of Bulgarian peasants resulted from their consumption of fermented milk products. At the time of Metchnikoff's scientific demonstration of lactic acid bacteria benefits, Henry Tissier[5], a French pediatrician, working independently observed that children with diarrhea had in their stools a low number of bacteria characterized by a peculiar, Y shaped morphology. These "bifid" bacteria were abundant in healthy children. He suggested that these bacteria could be administered to patients with diarrhea to help restore a healthy gut flora.

Probiotics frequently are isolated from human and animal gut. Dead bacteria, products derived from bacteria, or end products of bacterial growth also may impart certain benefits, but these derivatives are not considered to be probiotics because they are not alive when administered.

Probiotics have been studied for both human and animal applications. Some of the positive effects of probiotics are: protection of host from intestinal infections, enhancement of immune system [6]. Many bacteria are not harmful for us. In fact, our bodies carry about 100,000,000,000,000 ($1x10^{12}$) mostly in our colon. The difficulties of identifying and classifying strains, has complicated research, since benefits may only belong to particular strains [7]. *Lactobacillus*, *Lactococcus*, *Saccharomyces cerevisiae* and *Bifidobacteria* have a significant effect on the inflammation disease, it also helps in raising the immune system efficiency and has a remarkable effect on IgA, they are most commonly used as human probiotics for peptic ulcer .The isolation of probiotic strains has a considerable importance, as a consequence of interest in the potential health-promoting properties.

The isolation of *Bifidobacterium* species from feces has assumed a considerable importance [8]. *Lactobacilli* are ubiquitous in environment and in humans they play a very significant role in the general health, identification of *lactobacilli* has been based on culture dependent methods [9]. *Saccharomyces cerevisiae* is classified as a "probiotic," or a microorganism that

when ingested may have a positive influence on the host's health. Probiotics may give their effects on the alimentary canal directly, or may modulate the immune system in a larger scope.

Probiotics used in treatment of peptic ulcer by playing a big role in immune response. The immune-modulating effect in animals occurs in two ways: (a) from the microbiota, in which the probiotic migrates along the wall of the intestine and is multiplied to a limited extension, or (b) the antigen released by the dead organisms are absorbed and thus stimulate the immune system [10] .

METHODOLGY

Experimental animals

48 male Sprague dawley rats (body weight 200–250 gram) were purchased from VACSERA, Egypt. The animals were maintained in separate cages 3 per group, under standardized environmental conditions. Following a 7-day acclimation period, rats were randomized into experimental and control groups for induction of gastric ulcer. they were deprived of food but had free access to water for 24 h before induction of indomethacin [11] .

Rats were divided into 4 groups for each strain

Group 1: Normal (Negative control).

Group 2: is ulcerated without treatment (Positive control).

Group 3: is ulcerated and treated by specific strain

Group 4: is Normal non ulcerated and treated by probiotic strain only.

Bacteria (Probiotics) preparation and activation

Lactobacillus rhamnosus (ATCC 7469) ,*Bifidobacterium bifidum* (EMCC: 1334), *Lactococcus lactis* (ATCC 11454) and *Saccharomyces cerevisiae* (ATCC 7754) slants were purchased from Cairo MIRCEN, Ain Shams University. The *Bifidobacterium* broth media was used for Bifidobacterium, MRS Broth for lactobacillus, M17 broth for *Lactococcus* and PDA broth for *Saccharomyces*.MRS is based on the formulations of de Man, Rogosa and Sharpe (MRS) [12.]

Bifidobacterium broth was prepared by suspending 78.65 grams in 1000 ml distilled water. Media was completely dissolved by gentle heating for a few minutes. After sterilization by autoclaving at 15l pressure for 15 minutes, bifidobacteria was activated by inoculating 1 ml of it in 50 ml the media and incubating for 24-48 hours under anaerobic conditions..

MRS Broth media was prepared (Acumedia, United States) by dissolving 55 g of the medium in one liter of purified water, mixing them thoroughly and autoclaving at 121ºC for 15 minutes. After that the activation of the bacteria by inoculating 1 ml of *Lactobacillus rhamnosus* in 50 ml MRS media and incubate for 24-48 hours under anaerobic conditions.

M17 Broth media was prepared (Difco, United States) by dissolving 37.25 g of the medium in one liter of purified water, mixing them thoroughly and autoclaving at 121ºC for 15 minutes. Cooled to 50°C.50 mL sterile 10% lactose solution was added and mixed well. After that the activation of the bacteria by inoculating 1 ml of *Lacto*coccus *lactis* in 50 ml M17 media and incubate for 24-48 hours under anaerobic conditions.

PDA Broth media was prepared (Difco , United States)) by dissolving 24 g of the medium in one liter of purified water, mixing them thoroughly and autoclaving at 121ºC for 15 minutes. To alter the reaction of the agar medium to pH 3.5, cool the base to 45-50°C and aseptically add an appropriate amount of sterile 10% tartaric acid to each liter of medium. Mix well. Do not reheat the medium. After that the activation of the bacteria by inoculating 1 ml of *Saccharomyces* in 50 ml MRS media and incubate for 24-48 hours under aerobic conditions.

Preparation of Indomethacin

Non-steroidal anti-inflammatory drugs (NSAID) are generally used in the pain management, fever and inflammation. However, NSAIDs have been linked with ulcer and in several animal experiments and are widely used in ulcer induction in vivo which is “indomethacin” in our study [13.]

In our experiment, indomethacin was prepared by adding 0.5 gm of Gum accacia to 250 gm of indomethin powder and solubilizing them in distilled water using the morter and pistle to produce a 500 ml suspension.

Ulcer induction

Indomethacin was used to induce ulcer as it act as an NSAID (Non-steroidal anti-inflammatory drug) of strong anti-ulcer properties. Gastric ulceration was induced in the animals according to the procedure described by Sayanti [14]. Briefly, rats were administered with a single oral dose of indomethacin 40 mg/kg body weight in 1 ml water. They were deprived of food but had free approach to water 24 hours prior to ulcer induction.

Animal treatment

The 4 group where were orally administered the 1 ml of the treatment for 7 constitutive days and they accessed to food and water ad libitum. The protocol complies with the guidelines of the National Institute of Health (NIH, 1985) for laboratory animal care and use. The bacterial dose was $6x10^8$ *cfu* according to McFarland standard

Blood Sample collection

Blood samples were taken from the rats’ eyes in EDTA-collecting tubes for making complete blood count (CBC). Serum was collected in the day 7 after sacrificing the animals in blood collecting tubes, then separation by centrifugation at 2000 rpm for 15 minutes at 4°C, aliquotted and stored at -80°C for further investigations (Creatine kinase total serum (CK), IgA).

Stomach excision and collection of gastric tissue

Tissue samples were collected after indomethacin induction and at day 7 after treatment to compare between them. The specimens for each rat were fixed in 10% neutral buffered formalin solution and dehydrated in a graded alcohol series. Each specimen were examined grossly for dissection and detection of ulcer.

RESULTS

Complete blood count

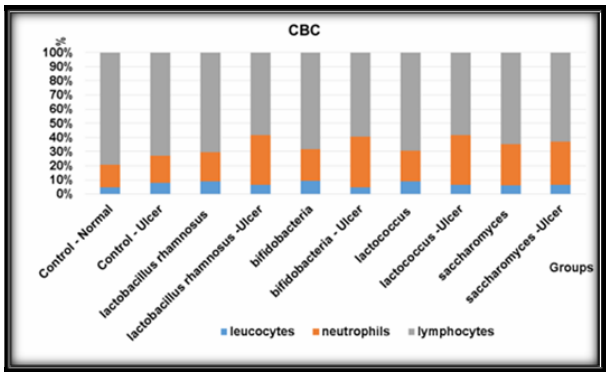

Fig. 1: Leukocytes and Lymphocytes had the highest level in control ulcer indicated the inflammation and they were decreased in probiotics with ulcer indicating decreased inflammation.

Creatine kinase total serum

The serum also used to detect the level of creatine kinase (CK) total test which also indicate inflammation, which is decreased in case of treatment by the used probiotics.

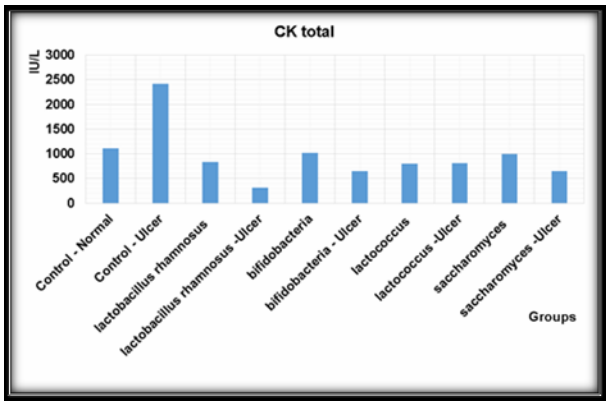

Fig. 2: Creatine kinase total serum had the highest level in control ulcer because the inflammation and were decreased in probiotics with ulcer indicating decreased inflammation.

IgA test

The secretory IgA is the major antibody in the intestinal mucosa, and after the treatment the percent of IgA was decreased as shown in case of treatment by the used probiotics. .

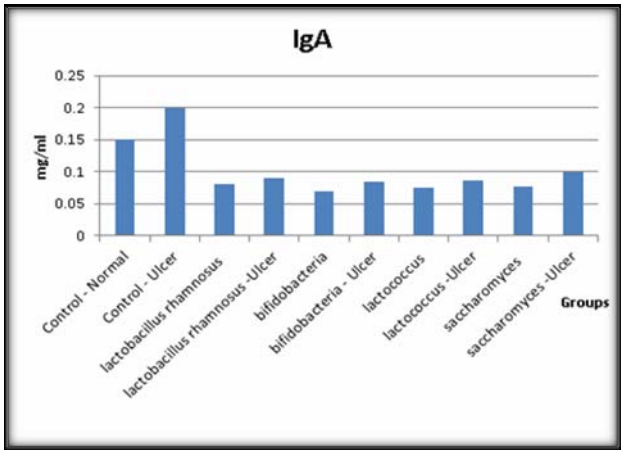

Fig. 3: IgA had the highest level in Control ulcer because of inflammation more than the normal and were decreased in probiotics with ulcer indicating decreased inflammation.

Histopathology

In this study, histopathological examination upon probiotics treatment showed enhanced ulcer healing with mild ulcerations compared to the ulcerated control as shown in Figures 4 to 13.

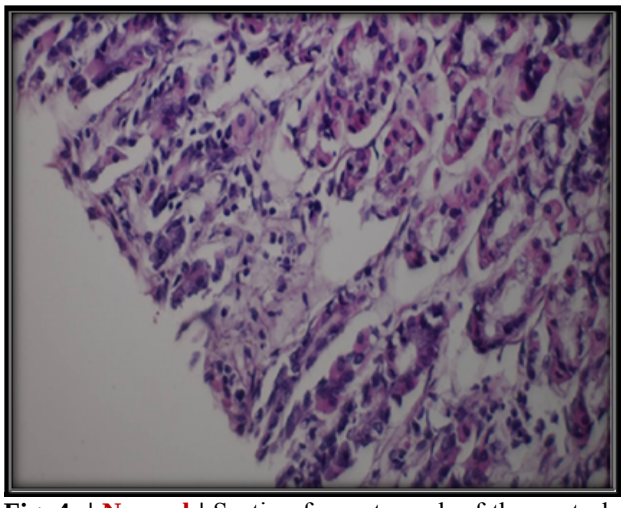

Fig. 4: | **Normal** | Section from stomach of the control negative group revealing normal histological structure with normal intact cells, gastric glands and mucosal lining.(H&E at 400).

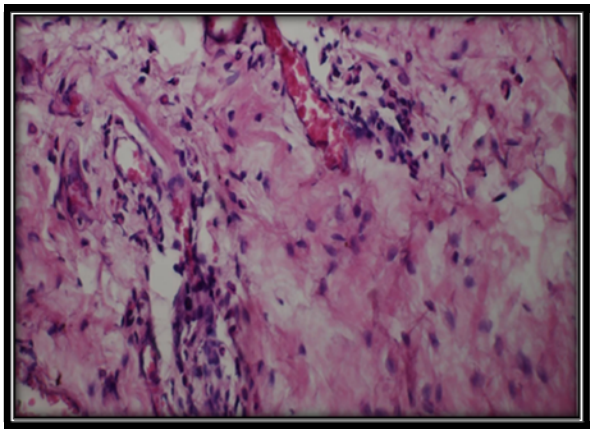

Fig. 5: | Ulcer | Photomicrograph of gastric ulcer revealing congestion of the submucosal blood vessels with inflammatory cells aggregation (H&E at 400).

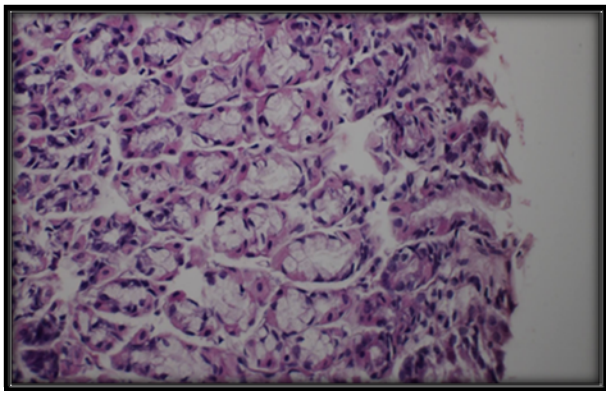

Fig. 6: | *lactobacillus rhamnosus normal* | Section from stomach shows normal histological structure with normal mucosal epithelium and gastric glands (H&E at 400).

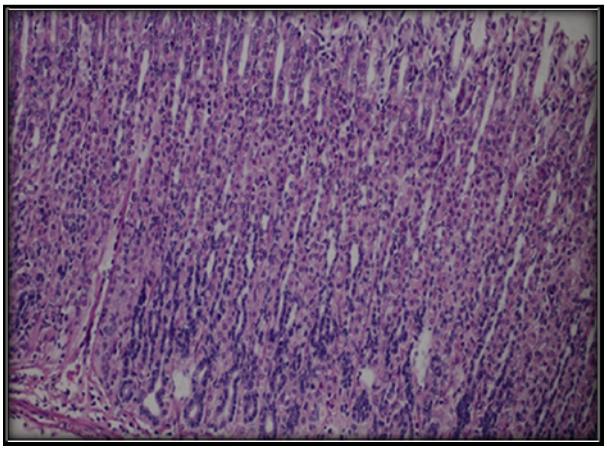

Fig. 7: |*lactobacillus rhamnosus* ulcerative | Stomach shows exhibiting marked improvement in the histological structure of the gastric mucosa with intact lining column epithelium and gastric glands revealing good healing process (H&E at 200).

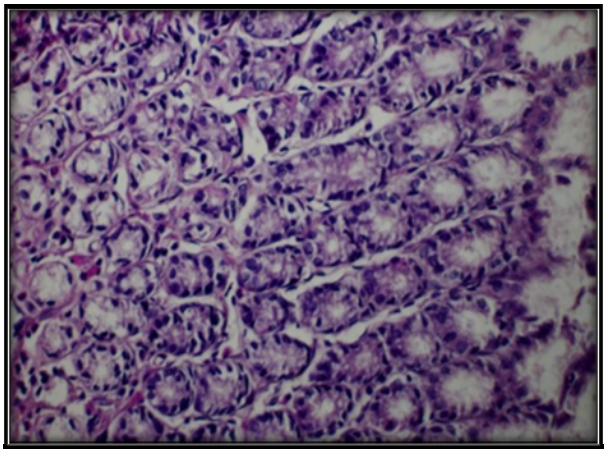

Fig. 8: | *lactococcus lactis* normal | Section from stomach shows normal histological structure with normal mucosal epithelium and gastric glands (H&E at 400).

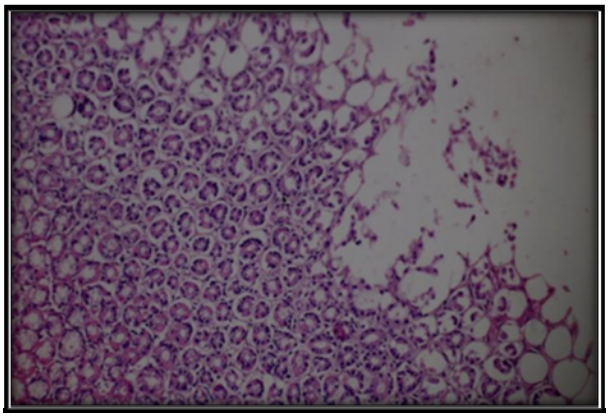

Fig. 9: | *lactococcus lactis* ulcerative| Stomach showed superficial gastric ulcer with tissue debris and mononuclear cells infiltration (H&E x400).

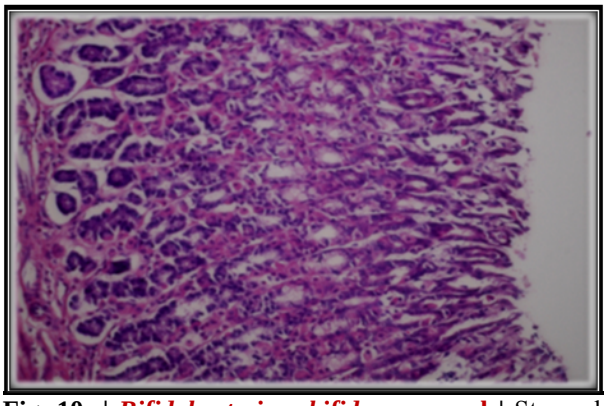

Fig. 10: | *Bifidobacterium bifidum* normal | Stomach showed normal histoarchitecture of gastric mucosa, gastric gland and tunica muscularis (H&E staining at 200X).

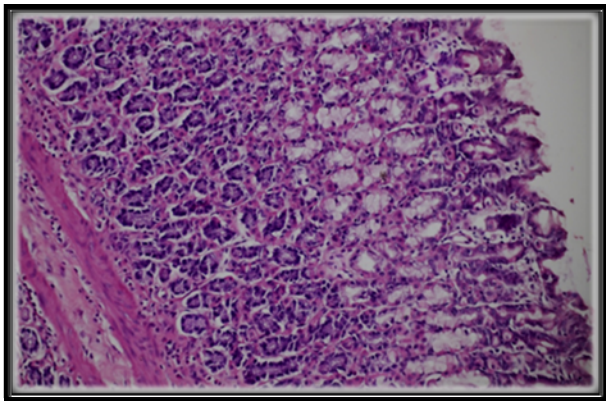

Fig. 11:| *Bifidobacterium bifidum* ulcerative | Stomach showed most of the gastric mucosa appears with intact lining epithelium, revealing marked improvement in the healing process (H&E staining at 200X).

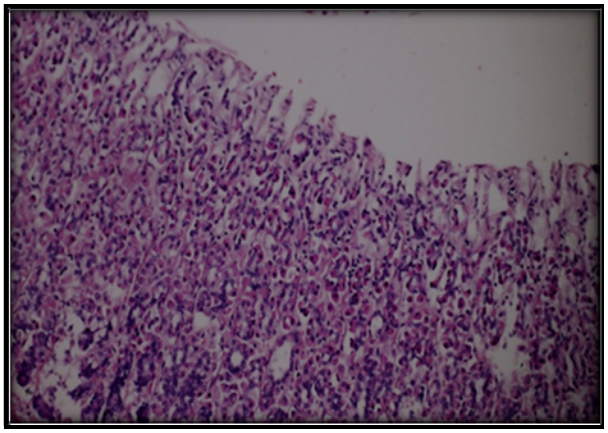

Fig. 12: |*Saccharomyces cerevisiae* normal| Stomach showed normal histopathological structure with normal mucosal epithelium, gastric glands(H&E x400)..

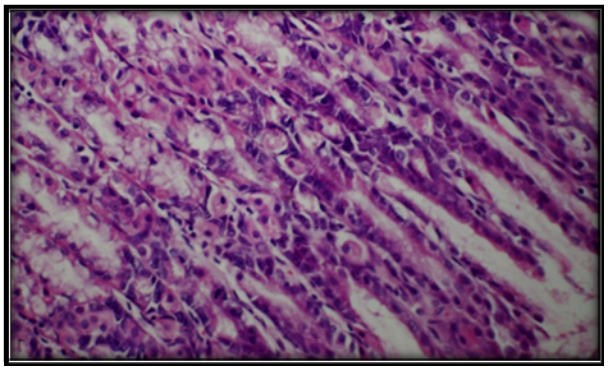

Fig. 13: |*Saccharomyces cerevisiae* ulcerative| Stomach showed revealing improvement in the pathological picture of gastric mucosa most of the lining epithelium was intact with normal gastric glands with few inflammatory cells in the lamina propria(H&E x400). .

DISCUSSION

The complete blood count (CBC) is a good, sensitive indicator of the organism health. WBCs estimates the body's ability to fight infections and diseases. WBCs count said to be increased in case of gastric ulceration [15]. According to previous studies, *lactobacillus rhamnosus* enhances gastric ulcer healing shown by decreasing WBCs count if used alone [16] and the same was found in some *in vitro* studies when *Bifidobacterium bifidum* was used [17]. Also, lactococcus lactis decreases WBCs count in gastric ulcer healing if used [18].

In addition to bacteria, certain yeasts, such as *Saccharomyces boulardii*, have been investigated and have shown potential therapeutic effects in a rat model of ibuprofen-induced gastric ulcer [19]. This yeast has neuraminidase activity, which removes sialic acid residues from the apical membranes of gastric epithelial cells.

In this study, our *Lactobacillus* and *Bifidobacterium* strains, causes a significant decrease in WBCs count by different degrees, compared to the positive control.

The current research indicates that supplementing diets with *S. cerevisiae* and other probiotics facilitates intestinal development, enhances immune functions, and increases physio-biochemical (e.g., CK) indexes to promote the performance of early-weaned pigs. Our results support previous reports involving rats [20.]
It was found that *Lactobacillus rhamnosus* [21] and Bifidobacterium[22] showed to decrease cellular apoptosis and enhance re-epithelization in induced gastric ulcer rats in vivo. Histopathological examination in some researches showed a great progression and enhanced healing effect upon lactobacillus administration[23]. It also enhances the immune cells recruitment to site of infection in order to accelerate the healing mechanism [24]. Our results support previous results.

REFERENCES

1. Khazaei, M., H., Salehi (2006).Protective effect of Falcaria vulgaris extract onethanol induced gastric ulcer in rat, Iranian J PharmacolTher 5:1–4.
2. Shristi, B., J.Neha, B.P.Indu,G. Rajesh, (2012). A review on some Indianmedicinal plants for antiulcer activity, J Sci Res Pharm 1:6–9.
3. Sabiua, S., T. Garubab, T., Sunmonuc, E., Ajania, A., Sulymana, I., Nuraina, A., Balogun. (2015). Indomethacin-induced gastric ulceration in rats: Protectiveroles of Spondiasmombin and Ficus exasperate.Elsevier Ireland Ltd.Toxicology Reports 2: 261–267.
4. Salminen S., C. Bouley, Boutron-Ruault, M.-C., J.H. Cummings, A. Franck, G.F.R. Gibson, E. Isolauri, M.-C. Moreau, M. Roberfroid, and I. Rowland (1998). Functional food science and

gastrointestinal physiology and function. Brit. J. Nutr. 80 (suppl. 1): S147–S171.
5. Tissier H. (1906). CR.Soc Biol, 60, 359 The Mayo Clinic. "Peptic Ulcer."http://www.mayoclinic.com/health/pepticulcer/DS00242/DSECTION=causes.
6. Sabit H., A. Farouk, O. Said, M. Yehia, A. Bishr (2011). Molecular characterization of bifidobacterium and lactobacillus and their role in modulating immune system and alleviating ulcerative colitis in mice.Global Journal of molecular science. 6 (1):9-14.
7. Herich R, M. Levkut (2002). Lactic acid bacteria, probiotics and immune system. Vet. Med. Czech, 47: 169– 180.
8. Arunchatam K, H.S. Gill and R.K. Chandra (2000). Enhancment of natural immune function by dietary consumption of Bifidobacterium lactis (HN019). Or . J. Covering joint Nutr .54(3):263-267.
9. Anukam K.C., O.O. Emmanuel , A. Ijeoma and R.Gregor (2005). 16S rRNA gene sequence and phylogenetic tree of lactobacillus species from the vagina of healthy Nigerian women. Afr. J. Biotechnol 4(11):1222-1227.
10. Havenaar R., S. Spanhaak (1994). Probiotics from an immunological point of view. Current Opinion in Biotechnology.
11. Dharmani, P.; C. De Simone and K. Chadee (2013). The Probiotic Mixture VSL#3 Accelerates Gastric Ulcer Healing by stimulating vascular endothelial growth factor .PLOS ONE,8(3).58671.
12. De Man, J.C., Rogosa, M. and Sharpe, M. E. (1960). A medium for the cultivation of lactobacilli. J Appl Bacteriol 23, 130–135.
13. Fehmi, O.; C. Ahmet; S. Halis; A. Ali; B. Yasin; H. Mesut; K. Cavit. (2006). Gastroprotective and antioxidant effects of usnic acid on indomethacin-induced gastric ulcer in rats. Journal of Ethnopharmacology. 103(1), 59–65.
14. Sayanti B., R.C. Susri, C. Subrata, K.B. Sandip (2007). Healing properties ofsome Indian medicinal plants against indomethacin-induced gastriculceration of rats, J Clin Biochem Nutr 41 (2) 106–114.
15. Emily, K.Y.; L. Yu; P.S. Helen; K.K. William; Y. Vivian; K.K. Emily; H.L. Wallace; C.Y. Patrick and C.H. Cho (2007). Probiotic Lactobacillus rhamnosus GG enhances gastric ulcer healing in rats. European Journal of Pharmacology, 565,171–179.
16. Ewaschuk; B. Julia; H. Diaz; L. Meddings; B. Diederichs; A. Dmytrash; J. Backer, M..L Langen and K.L. (2008). Madsen Am J Physiol Gastrointest Liver Physiol 295: 1025–1034.
17. Lee, C.J.; L.G. Chen; W.L. Liang and C.C. Wang (2010). Anti-inflammatory effects of Punica granatum Linne in vitro and in vivo. Food Chemistry, 118(2), 315–322.
18. Virchenko OV;Falalyeyeva TM; Beregova TV; Spivak MY; Lazarenko LM; Demchenko OM (2015). Effects of mono-, poly- and composite probiotics on the ulceration caused by restraint stress. Fiziol Zh ,61(1) :35-41.
19. Flatley EA; Wilde AM,;Nailor MD;J Gastrointestin (2015). Saccharomyces boulardii for the prevention of hospital onset Clostridium difficile infection. Liver Dis., 24(1):21-4.
20. Lee DL; Kuo TY; Chen MC; Tang TY; Liu FH, Weng CF (2006) .Expression of porcine epidermal growth factor in Pichia pastoris and its biology activity in early-weaned piglets. Life Sci 78:649–654.
21. Mohammed, W.; S. Cruickshank; A. McBain and C. O'Neill (2015). Lactobacillus rhamnosus GG Lysate Increases Re-Epithelialization of Keratinocyte Scratch Assays by Promoting Migration. Scientific Reports, 5(1), 16147.
22. Hoarau, C.; C. Lagaraine; L. Martin; F. Velge-Roussel and Y. Lebranchu (2006). Supernatant of Bifidoacterium breve induces dendritic cell maturation, activation, and survival through a Toll-like receptor 2 pathway. J Allergy Clin Immunol. 117:696–702.
23. Sarowska, J.; I. Choroszy-Król; B. Regulska-Ilow; M. Frej-Mądrzak and A. Jama-Kmiecik (2013). The therapeutic effect of probiotic bacteria on gastrointestinal diseases. Advances in Clinical and Experimental Medicine: Official Organ Wroclaw Medical University, 22(5), 759–66.
24. Ismail, T.; P. Sestili and S. Akhtar (2012). Pomegranate peel and fruit extracts: A review of potential anti-inflammatory and anti-infective effects. Journal of Ethnopharmacology, 143(2), 397–405.