MEDICAL SCIENCE

Modulation of neurotransmitter signaling along the Microbiota–Gut–Brain Axis by prebiotics, probiotics and synbiotics in peptic ulcerative rats

Huda A Al Doghaither[1], **Fares K Khalifa**[2*]

To Cite:
Al-Doghaither HA, Khalifa FK. Modulation of neurotransmitter signaling along the Microbiota–Gut–Brain Axis by prebiotics, probiotics and synbiotics in peptic ulcerative rats. *Medical Science* 2023; 27: e218ms3018. doi: https://doi.org/10.54905/disssi/v27i135/e218ms3018

Authors' Affiliation:
[1]Associate Professor, Biochemistry Department, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia
Email: haldoghaither@kau.edu.sa
ORCID: 0000-0002-6192-8326
[2]Professor, Biochemistry Department, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia/Department of Biochemistry and Nutrition, Faculty of Women for Arts, Science and Education, Ain Shams University, Cairo, Egypt
Email: fkkhalifa@kau.edu.sa
ORCID: 0000-0002-6320-2297

[*]**Corresponding Author**
Professor, Biochemistry Department, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia/Department of Biochemistry and Nutrition, Faculty of Women for Arts, Science and Education, Ain Shams University, Cairo,
Egypt
Email: fkkhalifa@kau.edu.sa
ORCID: 0000-0002-6320-2297

Peer-Review History
Received: 18 April 2023
Reviewed & Revised: 22/April/2023 to 02/May/2023
Accepted: 04 May 2023
Published: 05 May 2023

Peer-review Method
External peer-review was done through double-blind method.

Medical Science
pISSN 2321–7359; eISSN 2321–7367

DISCOVERY
SCIENTIFIC SOCIETY

ABSTRACT

Background: Normal brain processes need a healthy gut containing various microorganisms. Probiotics (Pro), prebiotics (Pre) and synbiotics (Syn) are able to prevent gut inflammation by restoring the makeup of the gut microbiome and introducing beneficial functionalities to gut microbial populations. Because of their functions in gut physiology and probable implications in the gastrointestinal and neurological systems pathology, neurotransmitters, such as norepinephrine, dopamine and serotonin, have lately attracted attention. In pathological conditions such as gastric ulcer (GU), neurotransmitter levels are dysregulated, resulting in a range of gastrointestinal symptoms. *Objectives:* To assess the effects of Pre, Pro and Syn on neurotransmitters that regulate gut microbiota and the gut–brain axis (GBA) under peptic ulceration circumstances. *Methods:* Fifty male rats were used in the study and were divided into groups as follows: Control group, ulcerative group and orally supplemented groups. Serum samples were used for measuring the levels of neurotransmitters in the blood. *Results:* Levels of serotonin, dopamine, gamma-aminobutyric acid (GABA), glutamate and norepinephrine were decreased in PU rats as compared to healthy rats. Treatment with Pro alone or in combination with Pre (PU + SynB) significantly improved the serum levels of neurotransmitters, inflammatory biomarkers and oxidative stress markers. *Conclusion:* Several neurological findings regarding the GBA reveal that the gut microbiota has strong bidirectional communication with the CNS and control the development and functions of the CNS, which, in turn, improves gut homeostasis.

Keywords: Gut microbiota, Gut–brain axis, Probiotics, Prebiotics, Synbiotics, Peptic ulcer, Neurotransmitters

1. INTRODUCTION

Neurotransmitters are not just hormone variables but also play a role in cell signaling. These are chemical compounds that function as "messengers" in nerve and synaptic communication. They attach to their matching receptors on the plasma membrane of peripheral and central cells. The gut–brain axis (GBA) is a complex and bidirectional link between the gut and the central nervous system (CNS) (Mayer, 2011; Iddrisu et al., 2022). It enables gut sensory visceral impulses to impact the CNS to control reflex and mood changes. The brain directs the signals to alter gut physiology (Heiss and Olofsson, 2019). Increasing data suggest that gut microbes have a crucial role on the GBA. As a result, the connection between the microbiota and the brain is commonly referred to as the microbiota/gut–brain axis (Mayer, 2011). The GBA is important for mediating between health and sickness. Since there is a bidirectional interaction, changes in the gut's bacterial nature may alter cerebral processes and vice versa (Cryan et al., 2020).

A peptic ulcer (PU) is one of the most prevalent and dangerous chronic upper gastrointestinal conditions. Despite advances in anti-ulcer medication, the incidence of recurrence remains high (Arakawa et al., 2012). The ulcer formation develops as a result of an imbalance between mucosal defense systems and harmful substances at the luminal surface of the stomach (Tarnawski et al., 2013). Ulcer healing demands different mechanisms to restore the balance between destructive and protective substances in the stomach. Several studies have shown that probiotics (Pro) may be utilized to heal PUs. When Pro and prebiotics (Pre) are supplied in sufficient concentrations, they yield health benefits to the host (Ashaolu, 2020).

Pro, Pre and Syn are being increasingly applied in different fields, including medicine and surgery. Pro's typical positive effects include rebuilding the gut flora and enhancing the intestinal and immunological balance (Azad et al., 2018). Moreover, Pro have been shown to have modulatory effects on CNS diseases, including the regulation of anxiety and depression-like symptoms (Abildgaard et al., 2017). Pre are non-digestible dietary fibers that improve host health by promoting the growth of gut microorganisms, particularly *Lactobacillus* and *Bifidobacterium* (Pandey et al., 2015). Syn are a combination of Pre and Pro. In terms of medicinal effectiveness, Syn possesses different properties, including antibacterial, anti-carcinogenic and anti-allergic actions. They also help reduce constipation and diarrhea by counteracting degradation processes in the colon. Syn action is based on the change of gut microbiota using probiotic bacteria and correctly chosen Pre as substrates. There are two known forms of Syn effect: (1) Increased survivability of probiotic bacteria; (2) Provision of particular health effects (Manigandan et al., 2012).

2. MATERIALS AND METHODS

Study Design

The study was conducted between August 2022 to January 2023. Fifty male Albino rats (Sprague-Dawely) were classified into five groups (10 in each), then treated as follows:

Group I (negative control): Healthy rats received basal diet.

Group II (PU): Ulcerated rat group (positive control).

Group III (PU + ProB): Ulcerated rats, fed basal diet and given daily oral probiotics supplementation of 1 mg/ml (200million CFU/ml).

Group IV (PU + PreB): Ulcerated rats, fed basal diet and given daily oral prebiotics supplementation of 1 mg/ml (200million CFU/ml).

Group V (PU + SynB): Ulcerated rats, fed basal diet and given daily oral supplementation of prebiotics and probiotics mixture (1 g/ml and 1 mg/ml, respectively).

Probiotics, prebiotics and synbiotics

Probiotics were obtained as natural products from California Gold Nutrition Co., USA, in the form of tablets, each tablet containing 0.5 mg (100million CFU) *Lactobacillus acidophilus* (LactoBif). Prebiotics were obtained as natural products from PreticXTM Prebiotic Co., USA, in the powdered form, xylo-Oligosaccharides (XOS) (Bifido Boost). Synbiotic was prepared as a mixture of preiotic and probiotics (*Lactobacillus acidophilus* + XOS) (SynB).

Experimental animals and induction of peptic ulcer (PU)

The study used adult male albino rats (Sprague-Dawley) weighing 105.7–113.5 g. Rats were kept in stainless steel cages within an air-conditioned animal house at 24°C, fed a basal diet and permitted water *ad libitum* throughout the experimental period (eight weeks). Peptic ulcer was induced by oral doses of aspirin (200 mg/kg per body weight/week).

Ethical committee approval

The study obtained the ethical clearance from the ethical committee at King Abdulaziz University No (304-22) before data collection.

Sample collection and biochemical assessment

After 56 days, following an overnight fast, blood was drawn from the hepatic portal vein of ether-anesthetized rats. Blood tubes were centrifuged at 5000 × g for 15 minutes at 24°C to separate the serum. Serum samples were collected in sterile plastic tubes and kept frozen at -20ºC for later biochemical testing. Kits for the assessment of dopamine, serotonin, glutamate, norepinephrine, nitric oxide (NO) and gamma-aminobutyric acid (GABA) were obtained from Bioassay Technology Laboratory (Shanghai, China). These kits use enzyme-linked immune sorbent assay (ELISA) based on the biotin double antibody sandwich technology. Kits for measuring superoxide dismutase (SOD), lipid peroxidation (MDA), catalase (CAT) and reduced glutathione (GSH) were purchased from Bio-vision, Milpitas, CA, USA. Kits for interleukin-6 (IL-6), tumor necrosis factor (TNF-α) and C-reactive protein (CRP) were purchased from Innova Biotech Co. Ltd, Beijing, China.

Statistical analysis

Data were statistically analyzed using SPSS software program (version 22.0, IBM Corp., Armonk, NY, USA). The results were shown as mean ± standard error (n = 10). The differences between mean values were determined using the one-way analysis of variance (ANOVA) test. P values less than 0.01 were considered statistically significant.

3. RESULTS

Table 1 shows the effect of Pre, Pro and Syn on the serum levels of the biogenic catecholamine neurotransmitters serotonin, dopamine and norepinephrine in the different rat groups. Serotonin, dopamine and norepinephrine levels were significantly ($p \leq 0.01$) decreased in PU rats compared to healthy rats. Meanwhile, an improvement was observed in Syn treated rats (PU + SynB) when compared to ulcerative rats. No significant ($p \leq 0.01$) changes in serum serotonin, norepinephrine and dopamine levels were observed among rats supplemented with PU + ProB or PU + PreB.

Table 1 Influence of different treatments on serum levels of biogenic amine neurotransmitters serotonin, dopamine and norepinephrine

Groups	Serotonin (ng/mL)	Dopamine (pg/ml)	Norepinephrine (pg/ml)
Control (C)	75.7±5.2^{a}	15.3±1.85^{a}	355.5±25.3^{a}
PU	40.5±2.5^{b}	5.75±0.50^{b}	199.5±6.5^{b}
PU + ProB	55.8±3.5^{c}	10.95±0.80^{c}	248.8±12.2^{c}
PU + PreB	51.3±2.1^{c}	10.35±1.05^{c}	239.5±10.3^{c}
PU + SynB	68.4±3.7^{d}	16.88±2.35^{a}	302.8±18.8^{d}

PU: Peptic ulcer, PreB: Prebiotics, ProB: Probiotics, SynB: Synbiotics.
There was no significant difference at $p < 0.01$ between means with the same alphabetic superscript (a, b, c and d) in the same column.

As in Table 2, reduced levels of GABA and glutamate were recorded in ulcerative rats (Group II) compared to the other groups. On the other hand, it was observed that administering SynB induced a significant ($p \leq 0.01$) improvement in both neurotransmitter levels compared to the PU group. No significant ($p \leq 0.01$) differences in GABA and glutamate levels were found between Pro (Group III) and Pre (Group IV) treated rats.

As in Table 3, administration of Pre and Pro significantly ($p \leq 0.01$) enhanced the serum levels of inflammatory biomarkers. Similar results were observed in serum TNF-α and CRP levels in rats administered with Pro in combination with Pre (SynB) compared with the PU group.

Table 2 Influence of different treatments on serum levels of amino acid neurotransmitters gamma-aminobutyric acid (GABA) and glutamate

Groups	GABA (pmol/ml)	Glutamate (mmol/L)
Control (C)	115.7±7.5[a]	11.30±1.1[a]
PU	82.9±4.1[b]	8.55±0.98[b]
PU + ProB	95.3±4.7[c]	9.60±0.85[b]
PU + PreB	89.2±3.6[d]	8.95±0.70[b]
PU + SynB	102.4±5.7[e]	10.50±0.25[a]

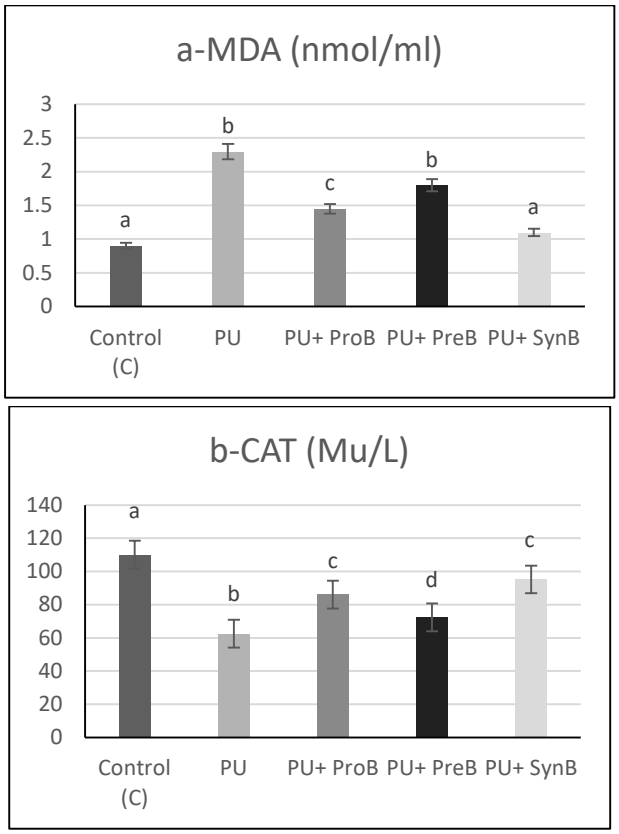
PU: Peptic ulcer, PreB: Prebiotics, ProB: Probiotics, SynB: Synbiotics.
There was no significant difference at $p < 0.01$ between means with the same alphabetic superscript (a, b, c and d) in the same column.

Table 3 Impact of various treatments on inflammatory biomarkers serum levels

Groups	TNF-α (pg/ml)	NO (µmol/l)	IL-6 (pg/ml)	CRP (ug/ml)
Control (C)	16.2± 1.8[a]	15.7 ±1.2 [a]	22.2 ± 1.1[a]	3.5 ±0.5 [a]
PU	39.5±5.7[b]	23.5 ±2.2 [b]	36.1±2.5[b]	6.2 ±0.8 [b]
PU + ProB	22.3±2.5[c]	19.1 ±1.7 [c]	29.8±3.1[c]	5.1 ±0.5 [c]
PU + PreB	23.1±2.7[c]	19.5 ±1.5 [c]	32.5±2.8[d]	5.0 ±0.9 [c]
PU + SynB	17.1±3.2[a]	20.3 ±1.8 [d]	24.9±1.8[a]	3.5 ±0.6 [a]

PU: Peptic ulcer, PreB: Prebiotics, ProB: Probiotics, SynB: Synbiotics.
There was no significant difference at $p < 0.01$ between means with the same alphabetic superscript (a, b, c and d) in the same column.

Oxidative stress is a critical pathogenic factor during peptic ulceration. Figure 1 (a-d) represents the effect of different treatments on serum oxidative stress biomarkers. Treatment with Pro (Group III) alone or in combination with Pre (PU + SynB) significantly ($p \leq 0.01$) improved the level of oxidative stress markers.



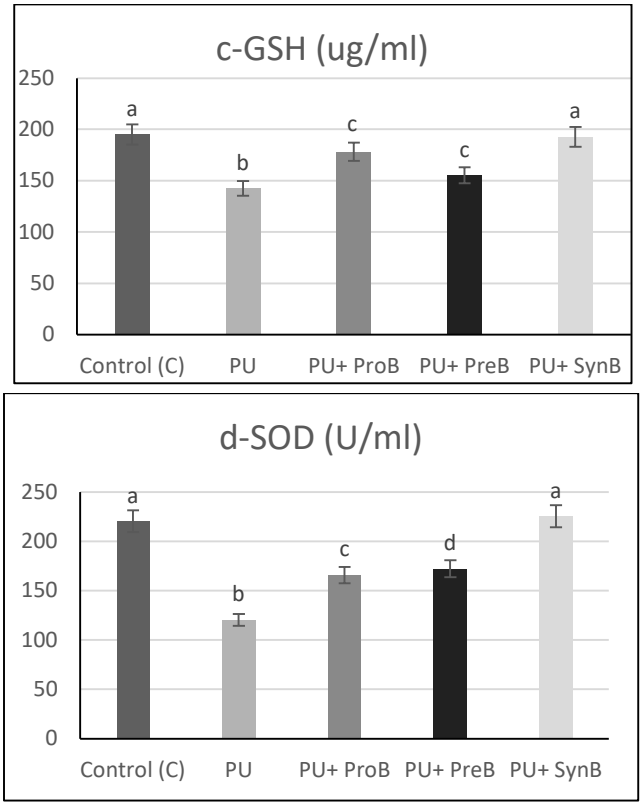


Figure 1 (a-d) Represents the oxidative stress biomarkers.

PU: Peptic ulcer, PreB: Prebiotics, ProB: Probiotics, SynB: Synbiotics.

There was no significant difference at $p < 0.01$ between means with the same alphabetic superscript (a, b, c and d).

4. DISCUSSION

The intestines are a complicated system with a dense and diverse micro flora known as gut microbiota. The gastrointestinal tract (GI) contains 100 trillion microorganisms (bacteria, viruses, fungi and protozoa) of at least 1,000 distinct species (Wang et al., 2017). These bacteria, as well as the genes linked with them and the environment that controls them, are all part of the "gut microbiome" (Valdes et al., 2018). The gut microbiota is thought to be a virtual endocrine organ, releasing chemicals capable of interacting with cells and triggering different reactions (Zhang and Davies, 2016). The intestinal microbiota serves a variety of activities, whose balance promotes the normal functioning of the body, immunity, metabolism and the creation of several neuroendocrine and neurotransmitter mediators (Woźniak et al., 2021).

The microbiota and the endocrine system interact bidirectionally, with hormone producing bacteria (e.g., serotonin and dopamine). Serotonin regulates GI motility and secretion. The results of the current study suggest that the microbiota has a role in controlling blood serotonin levels (Sjögren et al., 2012). The GBA is a bidirectional communication system that connects the central nervous system with the gastrointestinal (GI) tract. The majority of previous studies have shown the importance of gut bacteria in neurotransmitter metabolism and GI illness (Agus et al., 2018). Furthermore, in the intestinal lumen, neurotransmitters seem to regulate epithelial interactions with bacteria. By acting on the intestinal mucosa, both norepinephrine and dopamine have modified the mucosal adhesion of bacterial pathogens, such as *Escherichia coli* (De-Vadder et al., 2018).

Yano et al., (2015) discovered that the gut microbiota increases serotonin production from colonic enteroendocrine cells (ECs). In addition, gut bacteria are reported to enhance colonic serotonin synthesis through the effect of short-chain fatty acids (SCFAs) on ECs (Reigstad et al., 2015). As a result, microbiomes may have an impact on serotonin-related GI illness symptoms. Although the human microbiota does not control norepinephrine or dopamine *in vivo*, there is mounting evidence that it has a role in host biosynthesis.

Asano and his team revealed that mice deprived of bacteria had considerably lower levels of norepinephrine in the cecal lumen and tissue (Asano et al., 2012). This showed that the microbiota has an effect on norepinephrine levels in the lumen, although it is unclear whether the bacteria created norepinephrine directly or modified host production. Similarly, a work by Tsavkelova et al., (2000) showed that numerous microbes, including *Bacillus cereus, Bacillus mycoides* and *Escherichia coli*, were capable of generating

dopamine. This host–microbiota interaction adds to the emerging understanding that the microbiota affects GI physiology and disease by communicating with the host cells.

Any disruption in host–microbiota communication may affect the incidence and development of disease. Nonsteroidal anti-inflammatory medicines (NSAIDs), which may produce a variety of adverse outcomes, including gastrointestinal injuries, are one of the key variables that can alter the makeup of the microbiota (Wang et al., 2021). Serum levels of biogenic catecholamine neurotransmitters (serotonin, dopamine and norepinephrine) and amino acid neurotransmitters (glutamate and gamma-aminobutyric acid) were lower in peptic ulcerative rats. Pre, Pro and Syn consumption improves neurotransmitter levels significantly.

Bottom-up CNS regulation by the microbiota seems to occur predominantly via neuroimmune and neuroendocrine pathways, often involving the vagus nerve (Singh et al., 2016). Many microbially produced compounds, including SCFAs, secondary bile acids and tryptophan metabolites, facilitate this communication (Yano et al., 2015). While some of these substances transit the intestinal barrier, enter systemic circulation and may cross the blood–brain barrier, they primarily interact with enteroendocrine cells (EECs), enterochromaffin cells (ECs) and the mucosal immune system.

It is unclear if these chemicals reach the brain regions directly or only trigger central reactions through long-distance neural communication via vagal and/or spinal afferents (Bravo et al., 2011). The microbiota may create or contribute to the creation of a range of neuroactive chemicals, including gamma-aminobutyric acid (Barrett et al., 2012), serotonin, norepinephrine and dopamine (Asano et al., 2012). The cells of the gut's endocrine system are involved in an essential mechanism through which gut bacteria and their metabolites interact with the brain. There are at least 12 distinct kinds of these cells, with many subtypes appearing as subgroups throughout the gut and carrying diverse combinations of chemicals (Furness et al., 2013).

EECs are found throughout the gut, interspersed among gut epithelial cells and contain over 20 distinct kinds of signaling chemicals, many of which are co-localized and co-released. When these molecules are released as a response to chemical or mechanical stimuli, they are able to enter the systemic circulation and reach the behavior centers in the CNS. In addition, they can act locally and stimulate the vagal terminals in the gut or liver to produce brain signals. In these cells, different receptors implicated in controlling satiety and hunger have been discovered, which are triggered by microbial metabolites, such as SCFAs (Martin et al., 2018).

A persistent change in the composition or function of the microbiota (dysbiosis) may affect visceral sensitivity, intestinal motility and permeability, as well as the immune response, encouraging a pro-inflammatory state (Arrieta et al., 2014). Such changes, particularly in the host's immunological and metabolic systems, may initiate or encourage the emergence of a variety of illnesses, including diabetes, obesity and neurological disorders (Lynch and Pedersen, 2016).

The present study found that PU induced a strong inflammatory reaction, as shown by a substantial increase in blood IL-6, CRP, NO and TNF levels. Moreover, aspirin exacerbated oxidative damage and disrupted antioxidant parameter levels, according to the findings of the levels of MDA, SOD, CAT and GSH, as aspirin increases the production of free radicals, thus disturbing cellular antioxidant defense systems, the result being gastrointestinal ulcers in the rat stomach (Durak et al., 2001).

In the last decades, functional oligosaccharides were employed as a viable alternative to antibiotics. Xylo-Oligosaccharides (XOSs) are the best-known functional oligosaccharides. Since XOSs are not digested by digestive enzymes, they reach the distal sections of the intestines and are absorbed by the GI microbiota, specifically probiotic bacteria that create SCFAs (Patel and Goyal, 2011). Meanwhile, Pre administration (PU + PreB) was shown to be efficient in lowering MDA levels and enhancing GSH content, as well as SOD and CAT activity, in rat serum.

These findings proposed that dietary supplementation with Pre in the form of XOSs, in combination with *Lactobacillus acidophilus*, could reduce the aspirin-induced oxidative stress by modulating the antioxidant defense system and thus benefit human health. In agreement with the current study results, Le et al., (2020) studied the influence of prebiotic addition to probiotic cultures and noticed a suppression of Caco-2 cell line growth following addition of XOSs to fermented soymilk by bacterium cultures of *L. rhamnoses*.

Pre have critical roles in metabolic processes related to immunomodulation. Gastrointestinal barrier disruption permits numerous inflammatory mediators to pass from the gut into the bloodstream, a process known as metabolic endotoxemia, which has been linked to the development of obesity and diabetes in mouse models (Cani et al., 2009). When Pre and subsequent SCFAs release operate, the gut barrier integrity associated with immunity may be improved. The immunomodulatory impact of prebiotics is controlled by the variety of microbiota in the human intestine.

Anaerobic prebiotic fermentation generates mostly SCFAs, which may affect the expression of genes involved in the synthesis of anti-inflammatory cytokines in epithelial tissue (Pretorius et al., 2018). The generation of SCFAs by the HGM from prebiotic

fermentation is critical for maintaining gut health, shape and function (Raman et al., 2013). The present research found that XOS prebiotics decreased TNF, IL-6 and CRP production in peptic ulcer pre-supplemented rats (PU + PreB). These results may explain XOSs' immunomodulatory properties, given that the use of XOSs may boost immunity and protect against inflammatory disorders.

The present investigation found that supplementing with Pro (PU + ProB) substantially decreased the levels of oxidative stress indicators. *Lactobacilli* strains from the human or mouse GI tracts were used *in vitro* research on their antioxidative capabilities, as it displayed the capacity to degrade H2O2, which required CAT enzyme activity (Zanoni et al., 2008). According to Wang et al., (2018), compared to ulcerative animals, Pro supplementation dramatically enhanced total serum SOD and GSH levels while decreasing serum MDA concentration. Many reasons have been proposed for probiotics' antioxidative activity. Treatment with *Bacillus subtilis* has been found to reduce the expression of antioxidative genes, such as glutathione reductase and xanthine oxidase (Lei et al., 2015). Additional approaches have included decreasing inflammatory enzymes and regulating mitochondria-mediated apoptotic pathways (Esposito et al., 2009).

Certain Pro has been shown to improve the activities of some antioxidative enzymes or modulate circulatory oxidative stress, hence protecting cells against carcinogen-induced damage (Kumar et al., 2010). Similar findings indicating the protection of Pro against oxidative stress were published by Nardone et al., (2010). Similarly, Saide and Gilliland, (2005) showed that most *Lactobacilli* species have oxygen free radical scavenging mechanisms that may reduce the danger of ROS generation during meal digestion. Furthermore, the metabolic activity of probiotic bacteria may show an antioxidative impact by scavenging oxidant chemicals or preventing their production in the gut (Azcárate-Peril et al., 2011). Certain *Lactobacilli* have antioxidative activity and may reduce the danger of ROS formation during meal digestion (Kapila et al., 2006).

Given that a Pro is mainly active in the small and large intestines and the activity of a Pre is primarily detected in the large intestine, the combination of Pre and Pro (Syn) yields a collaborative outcome (Hamasalim, 2016). Pre are primarily employed as a selective medium for Pro strain development, fermentation and intestinal transit. Meanwhile, Pre and Pro microbes develop greater tolerance to environmental factors, such as pH and temperature in a specific organism's gut (Sekhon and Jairath, 2010). This combination (Syn) produces a viable microbiological dietary supplement, thus maintaining a suitable environment that has a good influence on the host's health. In addition, it preserves the intestinal bio structure, forms beneficial microbiota and suppresses the possible pathogens present in the GI tract (Scavuzzi et al., 2014). Moreover, Syn lowers the quantities of unwanted metabolites and inactivates nitrosamines and cancer-causing chemicals. Also, their usage increases the amounts of ketones, SCFAs, methyl acetates, and carbon disulfides, which are similarly beneficial for the host's health (Manigandan et al., 2012).

The present research found that Pro substantially decreased the release of TNF and IL-6. The benefits of *Lactobacillus acidophilus* Pro were connected with epithelial barrier modulation and normalization. As compared to the PU group, *Lactobacillus acidophilus* supplementation resulted in a slight but considerable improvement in stomach function. Pro (*Bifidobacterium bifidum* and *Lactobacillus acidophilus*) treatment reduced plasma IL-6 and TNF levels in elderly rats (Yang et al., 2020).

Pro has also been linked to better brain function and the prevention of neurological illnesses (Kinney et al., 2018; Li et al., 2020). Moreover, they greatly boost the beta microbiota diversity in the gut, Bifidobacterium proliferation and other anti-inflammatory bacteria in rats, indicating that Pre may have an influence on brain function via the GBA. On the other hand, Syn supplementation, which is a combination of selected Pro bacteria and Pre (with potentially synergistic effects), has been demonstrated to have health benefits, such as immune system regulation and anti-inflammatory (Kazemi et al., 2020), anti-depressant (Vaghef-Mehrabany et al., 2016) and antioxidant effects (Zheng et al., 2019).

The present research discovered that using Syn for eight weeks may reduce oxidative stress markers such as CRP, IL-6 and NO. The findings confirm the previously established relationship between Syn supplementation and reduced oxidative stress in cardiovascular illness (Vasquez et al., 2019) and neurological disease (Ton et al., 2020). Syn was also used to promote the growth of particular endogenous bacterial strains found in the gastrointestinal system (Gourbeyre et al., 2011; De-Vrese and Schrezenmeir, 2008). Given the vast number of conceivable mixtures, the use of Syn to modulate gut microbiota in humans appears to have great potential.

5. CONCLUSION

The results of the current study indicate that probiotics may be effective in the treatment of peptic ulcer and other digestive disorders. Prebiotics might be used as a substitute for probiotics or as supplementary assistance. The production of bio therapeutic formulations comprising both proper bacteria strains and synergistic prebiotic appears to boost the probiotics effect in the small intestine. The enhanced probiotics may be significantly more effective, with a stronger protecting and stimulatory effect than their

individual components. Future research is required to understand the mechanisms of action of these components, which may benefit human health.

Author Contribution

Dr Huda Al Doghaither wrote the manuscript and did the statistical analysis.
Dr Fares Khalifa designed the study and did the practical work.

Ethics committee approval

The study obtained the ethical clearance from the ethical committee at King Abdulaziz University No (304-22) before data collection.

Informed consent

Not applicable

Funding

This study has not received any external funding.

Conflict of interest

The authors declare that there is no conflict of interests.

Data and materials availability

All data sets collected during this study are available upon reasonable request from the corresponding author.

REFERENCES AND NOTES

1. Abildgaard A, Elfving B, Hokland M, Wegener G, Lund S. Probiotic treatment reduces depressive-like behavior in rats independently of diet. Psychoneuroendocrinology 2017; 79: 40-48. doi: 10.1016/j.psyneuen.2017.02.014
2. Agus A, Planchais J, Sokol H. Gut microbiota regulation of tryptophan metabolism in health and disease. Cell Host Microbe 2018; 23(6):716-724. doi: 10.1016/j.chom.2018.05.003
3. Arakawa T, Watanabe T, Tanigawa T, Tominaga K, Fujiwara Y, Morimoto K. Quality of ulcer healing in gastrointestinal tract: Its pathophysiology and clinical relevance. World J Gastroenterol 2012; 18(35):4811-4822. doi: 10.3748/wjg.v18.i35.4811
4. Arrieta MC, Stiemsma LT, Amenyogbe N, Brown EM, Finlay B. The intestinal microbiome in early life: Health and disease. Front Immunol 2014; 5:427. doi: 10.3389/fimmu.2014.00427
5. Asano Y, Hiramoto T, Nishino R, Aiba Y, Kimura T, Yoshihara K, Koga Y, Sudo N. Critical role of gut microbiota in the production of biologically active, free catecholamines in the gut lumen of mice. Am J Physiol Gastrointest Liver Physiol 2012; 303(11):G1288-95. doi: 10.1152/ajpgi.00341.2012
6. Ashaolu TJ. Immune boosting functional foods and their mechanisms: A critical evaluation of probiotics and prebiotics. Biomed Pharmacother 2020; 130:110625.
7. Azad MAK, Sarker M, Li T, Yin J. Probiotic species in the modulation of gut microbiota: An overview. Biomed Res Int 2018; 9478630. doi: 10.1155/2018/9478630
8. Azcárate-Peril MA, Sikes M, Bruno-Bárcena JM. The intestinal microbiota, gastrointestinal environment and colorectal cancer: A putative role for probiotics in prevention of colorectal cancer? Am J Physiol Gastrointest Liver Physiol 2011; 301(3):G401-24. doi: 10.1152/ajpgi.00110.2011
9. Barrett E, Ross RP, O'Toole PW, Fitzgerald GF, Stanton C. γ-Aminobutyric acid production by culturable bacteria from the human intestine. J Appl Microbiol 2012; 113(2):411-417. doi: 10.1111/j.1365-2672.2012.05344.x
10. Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, Dinan TG, Bienenstock J, Cryan JF. Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proc Natl Acad Sci U S A 2011; 108(38):16050-16055. doi: 10.1073/pnas.1102999108
11. Cani PD, Possemiers S, Wiele TV, Guiot Y, Everard A, Rottier O, Geurts L, Naslain D, Neyrinck A, Lambert DM, Muccioli GG, Delzenne NM. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut 2009; 58(8):1091-1103. doi: 10.1136/gut.2008.165886

12. Cryan JF, O'Riordan KJ, Sandhu K, Peterson V, Dinan TG. The gut microbiome in neurological disorders. Lancet Neurol 2020; 19(2):179-194. doi: 10.1016/S1474-4422(19)30356-4
13. De-Vadder F, Grasset E, Mannerås Holm L, Karsenty G, Macpherson AJ, Olofsson LE, Bäckhed F. Gut microbiota regulates maturation of the adult enteric nervous system via enteric serotonin networks. Proc Natl Acad Sci U S A 2018; 115(25):6458-6463. doi: 10.1073/pnas.1720017115
14. De-Vrese M, Schrezenmeir J. Probiotics, prebiotics and synbiotics. Adv Biochem Eng Biotechnol 2008; 111:1-66. doi: 10.1007/10_2008_097
15. Durak I, Karaayvaz M, Cimen MY, Avci A, Cimen OB, Büyükkoçak S, Oztürk HS, Ozbek H, Kaçmaz M. Aspirin impairs antioxidant system and causes peroxidation in human erythrocytes and guinea pig myocardial tissue. Hum Exp Toxicol 2001; 20(1):34-37. doi: 10.1191/096032701674627721
16. Esposito E, Iacono A, Bianco G, Autore G, Cuzzocrea S, Vajro P, Canani RB, Calignano A, Raso GM, Meli R. Probiotics reduce the inflammatory response induced by a high-fat diet in the liver of young rats. J Nutr 2009; 139(5):905-911. doi: 10.3945/jn.108.101808
17. Furness JB, Rivera LR, Cho HJ, Bravo DM, Callaghan B. The gut as a sensory organ. Nat Rev Gastroenterol Hepatol 2013; 10(12):729-740. doi: 10.1038/nrgastro.2013.180
18. Gourbeyre P, Denery S, Bodinier M. Probiotics, prebiotics and synbiotics: Impact on the gut immune system and allergic reactions. J Leukoc Biol 2011; 89(5):685-695. doi: 10.1189/jlb.1109753
19. Hamasalim HJ. Synbiotic as feed additives relating to animal health and performance. Adv Microbiol 2016; 6:288-302. doi: 10.4236/aim.2016.64028
20. Heiss CN, Olofsson LE. The role of the gut microbiota in development, function and disorders of the central nervous system and the enteric nervous system. J Neuroendocrinol 2019; 31(5):e12684. doi: 10.1111/jne.12684
21. Iddrisu I, Soumyakrishnan S, Joseph AA, Junhuan X, Boakai KR, Sreepriya M, Olufemi SA. Modulatory effect of gut microbiota on the gut-brain, gut-bone axes and the impact of Cannabinoids. Metabolites 2022; 12(12):1247. doi: 10.3390/metabo12121247
22. Kapila S, Vibha, Sinha PR. Antioxidative and hypocholesterolemic effect of Lactobacillus casei ssp casei (biodefensive properties of lactobacilli). Indian J Med Sci 2006; 60(9):361-370.
23. Kazemi A, Soltani S, Ghorabi S, Keshtkar A, Daneshzad E, Nasri F, Mazloomi SM. Effect of probiotic and synbiotic supplementation on inflammatory markers in health and disease status: A systematic review and meta-analysis of clinical trials. Clin Nutr 2020; 39(3):789-819. doi: 10.1016/j.clnu.2019.04.004
24. Kinney JW, Bemiller SM, Murtishaw AS, Leisgang AM, Salazar AM, Lamb BT. Inflammation as a central mechanism in Alzheimer's disease. Alzheimers Dement (N Y) 2018; 4:575-590. doi: 10.1016/j.trci.2018.06.014
25. Kumar M, Kumar A, Nagpal R, Mohania D, Behare P, Verma V, Kumar P, Poddar D, Aggarwal PK, Henry CJ, Jain S, Yadav H. Cancer-preventing attributes of probiotics: An update. Int J Food Sci Nutr 2010; 61(5):473-496. doi: 10.3109/09637480903455971
26. Le B, Ngoc APT, Yang SH. Synbiotic fermented soymilk with Weissella cibaria FB069 and xylooligosaccharides prevents proliferation in human colon cancer cells. J Appl Microbiol 2020; 128(5):1486-1496. doi: 10.1111/jam.14551
27. Lei K, Li YL, Wang Y, Wen J, Wu HZ, Yu DY, Li WF. Effect of dietary supplementation of Bacillus subtilis B10 on biochemical and molecular parameters in the serum and liver of high-fat diet-induced obese mice. J Zhejiang Univ Sci B 2015; 16(6):487-495. doi: 10.1631/jzus.B1400342
28. Li W, Guo J, Shen Y, Huang L, Leng B, Fan D, Shui L, Chen C. Probiotics, prebiotics and synbiotics for the treatment of dementia: Protocol for a systematic review. Medicine (Baltimore) 2020; 99(5):e18608. doi: 10.1097/MD.0000000000018608
29. Lynch SV, Pedersen O. The Human intestinal microbiome in health and disease. N Engl J Med 2016; 375(24):2369-2379. doi: 10.1056/NEJMra1600266
30. Manigandan T, Mangaiyarkarasi SP, Hemaltha R, Hemaltha VT, Murali NP. Probiotics, prebiotics and synbiotics- A review. Biomed Pharmacol J 2012; 5(2):295-304. http://biomedpharmajournal.org/?p=2511
31. Martin CR, Osadchiy V, Kalani A, Mayer EA. The brain-gut-microbiome axis. Cell Mol Gastroenterol Hepatol 2018; 6(2):133-148. doi: 10.1016/j.jcmgh.2018.04.003
32. Mayer EA. Gut feelings: The emerging biology of gut-brain communication. Nat Rev Neurosci 2011; 12(8): 453-466. doi: 10.1038/nrn3071
33. Nardone G, Compare D, Liguori E, Di Mauro V, Rocco A, Barone M, Napoli A, Lapi D, Iovene MR, Colantuoni A. Protective effects of *Lactobacillus paracasei* F19 in a rat model of oxidative and metabolic hepatic injury. Am J Physiol Gastrointest Liver Physiol 2010; 299(3):G669-76. doi: 10.1152/ajpgi.00188.2010
34. Pandey KR, Naik SR, Vakil BV. Probiotics, prebiotics and synbiotics- a review. Probiotics, prebiotics and synbiotics -a review. J Food Sci Technol 2015; 52(12):7577-7587. doi: 10.1007/s13197-015-1921-1

35. Patel S, Goyal A. The current trends and future perspectives of prebiotics research: A review. 3 Biotech 2012; 2(2):115-125. doi: 10.1007/s13205-012-0044-x
36. Pretorius R, Prescott SL, Palmer DJ. Taking a prebiotic approach to early immunomodulation for allergy prevention. Expert Rev Clin Immunol 2018; 14(1):43-51. doi: 10.1080/1744666X.2018.1411191
37. Raman M, Ambalam P, Kondepudi KK, Pithva S, Kothari C, Patel AT, Purama RK, Dave JM, Vyas BR. Potential of probiotics, prebiotics and synbiotics for management of colorectal cancer. Gut Microbes 2013; 4(3):181-192. doi: 10.4161/gmic.23919
38. Reigstad CS, Salmonson CE, Rainey JF 3rd, Szurszewski JH, Linden DR, Sonnenburg JL, Farrugia G, Kashyap PC. Gut microbes promote colonic serotonin production through an effect of short-chain fatty acids on enterochromaffin cells. FASEB J 2015; 29(4):1395-1403. doi: 10.1096/fj.14-259598
39. Saide JA, Gilliland SE. Antioxidative activity of lactobacilli measured by oxygen radical absorbance capacity. J Dairy Sci 2005; 88(4):1352-1357. doi: 10.3168/jds.S0022-0302(05)72801-0
40. Scavuzzi BM, Henrique FC, Miglioranza LHS, Simão ANC, Dichi I. Impact of prebiotics, probiotics and synbiotics on components of the metabolic syndrome. Ann Nutr Disord Ther 2014; 1:1009. https://austinpublishinggroup.com/nutritional-disorders/fulltext/andt-v1-id1009.pdf
41. Sekhon BS, Jairath S. Prebiotics, probiotics and synbiotics: An overview. J Pharm Educ Res 2010; 1:13-36.
42. Singh V, Roth S, Llovera G, Sadler R, Garzetti D, Stecher B, Dichgans M, Liesz A. Microbiota dysbiosis controls the neuroinflammatory response after stroke. J Neurosci 2016; 36(28):7428-7440. doi: 10.1523/JNEUROSCI.1114-16.2016
43. Sjögren K, Engdahl C, Henning P, Lerner UH, Tremaroli V, Lagerquist MK, Bäckhed F, Ohlsson C. The gut microbiota regulates bone mass in mice. J Bone Miner Res 2012; 27(6):1357-1367. doi: 10.1002/jbmr.1588
44. Tarnawski A, Ahluwalia A, Jones MK. Gastric cytoprotection beyond prostaglandins: Cellular and molecular mechanisms of gastroprotective and ulcer healing actions of antacids. Curr Pharm Des 2013; 19(1):126-132. doi: 10.2174/13816128130117
45. Ton AMM, Campagnaro BP, Alves GA, Aires R, Côco LZ, Arpini CM, Oliveira TGE, Campos-Toimil M, Meyrelles SS, Pereira TMC, Vasquez EC. Oxidative stress and dementia in Alzheimer's patients: Effects of synbiotic supplementation. Oxid Med Cell Longev 2020; 2020:2638703. doi: 10.1155/2020/2638703
46. Tsavkelova EA, Botvinko IV, Kudrin VS, Oleskin AV. Detection of neurotransmitter amines in microorganisms with the use of high-performance liquid chromatography. Dokl Biochem 2000; 372(1-6):115-117.
47. Vaghef-Mehrabany E, Homayouni-Rad A, Alipour B, Sharif SK, Vaghef-Mehrabany L, Alipour-Ajiry S. Effects of probiotic supplementation on oxidative stress indices in women with rheumatoid arthritis: A randomized double-blind clinical trial. J Am Coll Nutr 2016; 35(4):291-299. doi: 10.1080/07315724.2014.959208
48. Valdes AM, Walter J, Segal E, Spector TD. Role of the gut microbiota in nutrition and health. BMJ 2018; 361:k2179. doi: 10.1136/bmj.k2179
49. Vasquez EC, Pereira TMC, Peotta VA, Baldo MP, Campos-Toimil M. Probiotics as beneficial dietary supplements to prevent and treat cardiovascular diseases: Uncovering their impact on oxidative stress. Oxid Med Cell Longev 2019; 2019:3086270. doi: 10.1155/2019/3086270
50. Wang B, Yao M, Lv L, Ling Z, Li L. The human microbiota in health and disease. Engineering 2017; 3(1):71-82. doi: 10.1016/J.ENG.2017.01.008
51. Wang X, Tang Q, Hou H, Zhang W, Li M, Chen D, Gu Y, Wang B, Hou J, Liu Y, Cao H. Gut microbiota in NSAID enteropathy: New insights from inside. Front Cell Infect Microbiol 2021; 11:679396. doi: 10.3389/fcimb.2021.679396
52. Wang Y, Guo Y, Chen H, Wei H, Wan C. Potential of Lactobacillus plantarum ZDY2013 and Bifidobacterium bifidum WBIN03 in relieving colitis by gut microbiota, immune and anti-oxidative stress. Can J Microbiol 2018; 64(5):327-337. doi: 10.1139/cjm-2017-0716
53. Woźniak D, Cichy W, Przysławski J, Drzymała-Czyż S. The role of microbiota and enteroendocrine cells in maintaining homeostasis in the human digestive tract. Adv Med Sci 2021; 66(2):284-292. doi: 10.1016/j.advms.2021.05.003
54. Yang X, Yu D, Xue L, Li H, Du J. Probiotics modulate the microbiota-gut-brain axis and improve memory deficits in aged SAMP8 mice. Acta Pharm Sin B 2020; 10(3):475-487. doi: 10.1016/j.apsb.2019.07.001
55. Yano JM, Yu K, Donaldson GP, Shastri GG, Ann P, Ma L, Nagler CR, Ismagilov RF, Mazmanian SK, Hsiao EY. Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. Cell 2015; 161(2):264-276. doi: 10.1016/j.cell.2015.02.047
56. Zanoni S, Pompei A, Cordisco L, Amaretti A, Rossi M, Matteuzzi D. Growth kinetics on oligo- and polysaccharides and promising features of three antioxidative potential probiotic strains. J Appl Microbiol 2008; 105(5):1266-1276. doi: 10.1111/j.1365-2672.2008.03860.x
57. Zhang LS, Davies SS. Microbial metabolism of dietary components to bioactive metabolites: Opportunities for new therapeutic interventions. Genome Med 2016; 8(1):46. doi: 10.1186/s13073-016-0296-x
58. Zheng HJ, Guo J, Jia Q, Huang YS, Huang WJ, Zhang W, Zhang F, Liu WJ, Wang Y. The effect of probiotic and

synbiotic supplementation on biomarkers of inflammation and oxidative stress in diabetic patients: A systematic review and meta-analysis of randomized controlled trials. Pharmacol Res 2019; 142:303-313. doi: 10.1016/j.phrs.2019.02.016