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ORIGINAL ARTICLE

Metabiotic extracted from *Bifidobacterium bifidum* modulates antioxidant capacity and inflammatory responses during peptic ulcer healing in male wistar rats: a preliminary study

Rika Almasyan[1], Parvaneh Jafari[1*], Ghasem Farjanikish[2], Hassan Noorbazargan[3]

[1]Department of Microbiology, Faculty of Science, Arak Branch, Islamic Azad University, Arak, Iran
[2]Department of Pathobiology, Faculty of Veterinary Medicine, Lorestan University, Khorram Abad, Iran
[3]Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

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ABSTRACT

Background and Objectives: Peptic ulcer disease is a multifactorial disease that affects up to 10% of people. The use of natural product remedies has received much attention for its treatment. In this research, the healing effect of metabiotic extracted from *Bifidobacterium bifidum* was investigated.

Materials and Methods: 45 male wistar rats were divided into 3 groups (Ctrl-, drug, and metabiotic), and stomach ulcers were induced by ethanol administration and treated by drug and metabiotic. The healing process was investigated on different days by histological analysis and qRT-PCR.

Results: The metabiotic increased IL-8 and PDGF expression and stimulated the recruitment of polymorphonuclear cells to the wound site. It caused a faster onset of the inflammation phase followed by the proliferation phase. The metabiotic increased the expression of SOD and GPx genes and the antioxidant capacity of the wound. The increase in EGF expression led to faster re-epithelization, which was evident in the wound closure process.

Conclusion: Metabiotic extracted from *B. bifidum* is a promising candidate for the treatment of PUD. It causes a faster onset of the inflammation phase. Improving the antioxidant status of the wound, causes a faster resolution of inflammation, which leads to the acceleration of the wound-healing process.

Keywords: *Bifidobacterium bifidum*; Metabiotic; Peptic ulcer; Inflammation; Antioxidant

INTRODUCTION

Peptic ulcer disease (PUD) is a common disorder of the digestive system. It is defined as a digestive tract injury that results in a mucosal break greater than 3-5 mm, with a visible depth reaching the submucosa (1). These ulcers mainly occur in the stomach and proximal duodenum, and 5 to 10% of people will experience them during their lifetime (2, 3). Among the most important causes of these ulcers are infection with *Helicobacter pylori* and the use of anti-inflammatory drugs (NSAIDs), smoking, and physiological stress. PUD can cause various complications such as bleeding, perforation, penetration into nearby organs, and obstruction of the digestive system (4, 5). In fact, gastric ulcer is the result of a

*Corresponding author: Parvaneh Jafari, Ph.D, Department of Microbiology, Faculty of Science, Arak Branch, Islamic Azad University, Arak, Iran. Tel: +98-9122267223 Fax: +98-2188033824 Email: p-jafari@iau-arak.ac.ir

tissue necrosis caused by mucosal ischemia and the formation of free radicals. Gastric ulcer is the result of tissue necrosis caused by mucosal ischemia and the formation of free radicals.

The healing process of stomach wounds is a complex, dynamic process that is orchestrated by immune cells, interleukins, and chemokines (6, 7). In the first minutes after creating a wound, the hemostasis phase begins. In this phase, a set of inactive zymogens are converted into active serine proteases and lead to platelet activation and fibrin clot formation (8, 9). Activated platelets release growth factors such as PDGF and inflammatory mediators such as IL-8 or CXCL8 (a strong attractant of neutrophils). During the first 72 hours after wound formation, the inflammatory phase initiate which is characterized by the entry of neutrophils and monocytes into the wound site. The release of leukotriene B and the binding of necrotic tissue cell compounds to DAMP receptors in phagocytic cells creates a signaling cascade that causes the production of pro-inflammatory cytokines such as TNFα, IL-lα, and IL-lβ (10, 11). During a phenomenon called a respiratory burst, phagocytic cells produce very large amounts of reactive oxygen species (ROS), which play a very important role as secondary messengers in the wound healing process. Balanced levels of ROS cause the proliferation and stimulation of growth factors production by fibroblasts so accelerating re-epithelization healing. Studies showed that ROS can be very important in the movement of progenitor cells (bone marrow) to the wound site. Furthermore, ROS-mediated transcription factor activation leads to the secretion of growth factors for autocrine/paracrine signaling in wound healing processes. Many enzymes involved in wound healing signaling pathways have sulfhydryl residues in their catalytic center. These enzymes are very sensitive to oxidative changes and become inactive by oxidation. Therefore, the increase in ROS level due to the imbalance in oxidative homeostasis creates a signaling network that leads to impaired wound healing (12-15).

Unfortunately, no specific cut-off has been determined for ROS and redox imbalance occurs when the capacity of endogenous antioxidants is not sufficient to clear ROS. Antioxidants are divided into two categories, enzymatic and non-enzymatic antioxidants (16, 17). Non-enzymatic antioxidants are low molecular weight compounds such as vitamin E, vitamin C, glutathione, and flavonoids. Superoxide dismutase (SOD), glutathione peroxidases (GPx), and catalase are among the most important antioxidant enzymes in biological systems. SOD is the most powerful antioxidant and the key factor in cell protection against superoxide anions. By dismutation, this enzyme converts superoxide anion into hydrogen peroxide, which is further catalyzed by GPx and catalase enzymes into water and oxygen (18-20).

Recently, the use of an antioxidant system has been considered a new strategy for the improvement of wound healing. In this regard, various natural compounds such as probiotics have been evaluated (21-24). The risk of using complete bacteria in immunocompromised people led us to investigate the possibility of using metabiotic in the treatment of PUD (25, 26). Metabiotics are useful bioactive substances secreted by probiotics that have received much attention recently. In this study, we investigated the effect of the metabiotic extracted from *B. bifidum* ATCC 29521 in the treatment of PUD caused by alcohol administration in the wistar rat model.

MATERIALS AND METHODS

Metabiotic preparation. *B. bifidum* ATCC 29521 used in this research was purchased from the Center of scientific and industrial research (Tehran, Iran). The probiotic properties of this bacteria and its active compounds in cell-free supernatant (metabiotic) were specified by Jafari et al. To prepare metabiotic, the bacterium was cultured in DeMan-Rogosa-Sharpe (MRS) broth (Merck, Germany) supplemented with 0.5 g/l L-cysteine (27). The culture was incubated at 37°C for 48 h and the biomass was separated by centrifugation at 6000 rpm for 20 minutes at 4°C. The isolated metabiotic was aliquoted into Eppendorf tubes and stored at -20°C until use.

Animals. 45 male Wistar rats (4 weeks old, weighing 250-300 g) were purchased from Pasteur Institute (Tehran, Iran). Rats were kept in the animal house of Kermanshah University of Medical Sciences under controlled conditions of temperature (23 ± 2°C), humidity (50 ± 10%), and 12/12 light/dark cycle. Animals had ad-libitum access to water and standard food (Behparvar Co.). All applied protocols were approved by the Committee on the Ethics of Animal Experiments of Lorestan University of Medical Sciences (Permit Number: IR.LUMS.REC.1398.230).

After 10 days of acclimatization, the rats were randomly divided into 3 groups. The groups included the negative control group (Ctrl-), the drug group (Drug), and the metabiotic group (Metabiotic). To create peptic ulcers, the rats were starved for 48 h with free access to water. Then 1 ml of 98% ethanol was orally gavaged to rats for 8 consecutive days (28).

The drug used for the treatment of peptic ulcers included sucralfate (2 mg), omeprazole (1 mg), and magnesium-aluminum (1 mg) (29, 30). The drugs were dissolved in 1 ml of PBS buffer and gavaged to the rats daily. The rats in metabiotic and ctrl- groups also received 1 ml of metabiotic and PBS buffer, respectively.

On days 1, 3, 7, 14 and 21-days post wounding (PW), 3 rats from each group were randomly selected and anesthetized by intraperitoneal injection of ketamine (75 mg/Kg) and xylazine (10 mg/Kg) (31). Then the animals were killed by cardiac puncture (32). Subsequently, the rats' stomachs were surgically removed and opened along greater curvature to evaluate the wound healing process.

Wound closure assay and histological analysis. In order to evaluate the closing process, photographs were taken of the wound on different days. Then the images were analyzed with the ImageJ program. The percentage of wound healing was calculated as fallow (33):

$$H = \frac{A_1 - A_x}{A_1} \times 100$$

A_1: ulcer size after one day

A_x: ulcer size after x day

H: percentage of healing

To evaluate the closing process, photographs were taken of the wound on different days. Then the images were analyzed with the ImageJ program. The percentage of wound healing was calculated as follow.

Subsequently, each wound sample was divided into two parts along the largest diameter. Half of the sample was fixed in 10% formaldehyde for 24 h and used for further histological analyses. The other half was placed in Ranse-free tubes and kept at -60°C for gene expression analysis (34).

Tissue samples fixed in formaldehyde were dehydrated in increasing concentrations of alcohol. The samples were clarified using a xylene solution. Finally, paraffin blocks were prepared and 5 µm thick sections were prepared from tissues using a microtome. After staining with hematoxylin and eosin (H & E), the tissues were evaluated microscopically in terms of immune cell infiltration and healing (35).

Quantitative real-time PCR (qRT-PCR). Tissue total RNA was extracted using RNX-Plus solution (Cinnaclon, Tehran, Iran) according to the manufacturer's instructions. RNA concentration and purity were determined by a spectrophotometer (Thermo Scientific NanoDrop™ 1000). Then WizScript™ cDNA Synthesis Kit was used for cDNA synthesis from 11 µg of RNA and cDNA concentration was measured spectrophotometrically. The sequence of primers used for amplification of gene fragments are shown in Table 1. Quantitative RT-PCR was assayed by the rotor gene 6000 corbette detection system and iQ5 detection system (Bio-Rad, USA). The thermal cycles were included primary activation stage at 95°C for 10 min, 40 cycles at 72°C for 10 seconds, and final extension at 62°C for 15 min. The Genes expression level was determined using the $2^{-\Delta\Delta Ct}$ formula.

Table 1. The sequence of primers used for Real-time PCR

Gene	Primer	5'→ 3' sequence
PDGF	Forward	AGCCAAGACACCTCAAACTC
	Reverse	TAAATAACCCTGCCCACACTC
IL-8	Forward	ACGAGAGTGATTGAGAGTGGAC
	Reverse	AACCCTCTGCACCCAGTTTTC
EGF	Forward	GCAAACAGAGAAGAGGACAG
	Reverse	CAACAAGTTCGTGACATCGT
GPX	Forward	CAGTTCGGACATCAGGAGAAT
	Reverse	AGAGCGGGTGAGCCTTCT
SOD	Forward	CGAGCATGGGTTCCATGTC
	Reverse	CTGGACCGCCATGTTTCTTAG
GAPDH	Forward	CACTCAGAAGACTGTGGATGG
	Reverse	ATACTTGGCAGGTTTCTCCAG

Statistical analysis. GraphPad Prism 8.01 program was used for statistical analyses and graphic drawings. The data were analyzed using one-way ANOVA, followed by the Tukey HSD multiple comparison test. Results are given as the mean ± standard error of mean (S.E.M.). A value of $p < 0.05$ was considered as significant.

RESULTS

Wound closure. The results showed that during the treatment process, there was a significant decrease in

wound area in treated groups (Table 2). Interestingly, the use of metabiotic accelerated the macroscopic process of wound healing during the early days of healing. From day seven onwards, the complete closure of the wound was obvious in this group. The use of drugs also improved wound closure. Macroscopic studies showed that in this group, the wound was almost closed after 14 days.

Histological analysis. Immediately after injury, a hemostatic plug is formed by blood constituents like platelets. The Chemoattractant which is released from a blood clot causes the transmigration of neutrophils across the endothelial cell wall of blood capillaries. Recruited neutrophils remove damaged cells and infectious agents in the wound site and initiate the inflammatory phase.

Our results showed that the number of polymorphonuclears in the treated groups reached the maximum on the first day (PW) and was significantly higher in the untreated group (Table 2 and Fig. 1). It seems that PMNs undergo apoptosis in the following days and their number decreases. A significant decrease in the number of PMN compared to the control group was observed from the third day onwards. This indicates a faster resolution of the inflammatory phase. So, it can be assumed that all of the treatments accelerated and intensified the inflammation phase.

A similar pattern was seen in the recruitment of monocyte/macrophage cells in such a way that their number reached the maximum within 3 days (PW). During the following days, a significant decrease in the number of these immune cells was observed. Interestingly, the peak number of macrophages in the metabiotic group was observed on the third day, which had a significant decrease in the following days compared to the drug group. In other words, the inflammation phase ended faster than the drug group, which was also associated with faster wound closure.

The number of fibroblasts in the metabiotic group reached the maximum value on the 7th day (PW). These cells cause the formation of granulation tissue and the deposition of collagen and elastin strands, which will strengthen and close the wound.

Gene expression assay by qRT-PCR. Our results showed that treatment with drugs and metabiotic significantly stimulated the expression of PDGF and EGF genes in the 7th and 14th days (PW) compared to the control group. Interestingly, metabiotic had a higher potential in stimulating EGF- expression than the drug-treated group (Table 3). The remarkable

Table 2. Infiltration of polymorphonuclear, macrophage, and fibroblasts peptic wound healing in rats with different treatment models.

	Day				
Group	**1**	**3**	**7**	**14**	**21**
Macrophages NO. (Mean ± SEM)					
Ctrl-	3.20 ± 0.37^{c}	12.00 ± 0.8^{4c}	25.20 ± 1.56^{a}	16.40 ± 1.29^{a}	11.00 ± 0.32^{a}
Drug	9.20 ± 0.73^{b}	29.20 ± 1.02^{a}	16.40 ± 0.81^{b}	10.60 ± 0.51^{b}	6.60 ± 0.68^{b}
Metabiotic	13.40 ± 1.69^{a}	19.20 ± 0.86^{b}	10.20 ± 1.07^{c}	6.8 ± 0.92^{c}	3.00 ± 0.45^{c}
Polymorphonuclear leukocytes NO. (Mean ± SEM)					
Ctrl-	36.80 ± 0.80^{b}	11.00 ± 0.32^{b}	27.00 ± 1.87^{a}	21.00 ± 0.89^{a}	13.20 ± 1.16^{a}
Drug	50.40 ± 1.86^{a}	37.20 ± 1.24^{a}	16.80 ± 1.63^{b}	8.60 ± 0.93^{b}	6.40 ± 0.71^{b}
Metabiotic	45.00 ± 2.34^{a}	29.20 ± 1.16^{a}	13.80 ± 0.58^{b}	9.80 ± 0.86^{b}	4.00 ± 0.45^{b}
Fibroblast NO. (Mean ± SEM)					
Ctrl-	0.14 ± 0.02^{b}	3.20 ± 0.58^{d}	11.00 ± 0.84^{d}	23.20 ± 1.28^{a}	23.80 ± 1.46^{b}
Drug	0.21 ± 0.03^{b}	26.80 ± 1.50^{b}	32.80 ± 1.32^{b}	20.80 ± 1.86^{a}	12.80 ± 1.43^{a}
Metabiotic	0.70 ± 0.10^{a}	34.80 ± 1.93^{a}	41.60 ± 2.32^{a}	19.20 ± 2.18^{a}	13.20 ± 0.80^{a}
Percentage of Wound closure (Mean ± SEM)					
Ctrl-	0.12 ± 0.01^{c}	33.33 ± 6.01^{c}	50.17 ± 2.89^{c}	88.50 ± 1.76^{b}	88.87 ± 3.25^{b}
Drug	0.35 ± 0.09b^{c}	60.14 ± 2.38^{b}	83.70 ± 3.42^{b}	98.50 ± 1.26^{a}	99.17 ± 0.83^{a}
Metabiotic	0.62 ± 0.09^{a}	82.33 ± 3.71^{a}	98.40 ± 0.17^{a}	100^{a}	100^{a}

Means with the same letter in a column are not significantly different from each other.

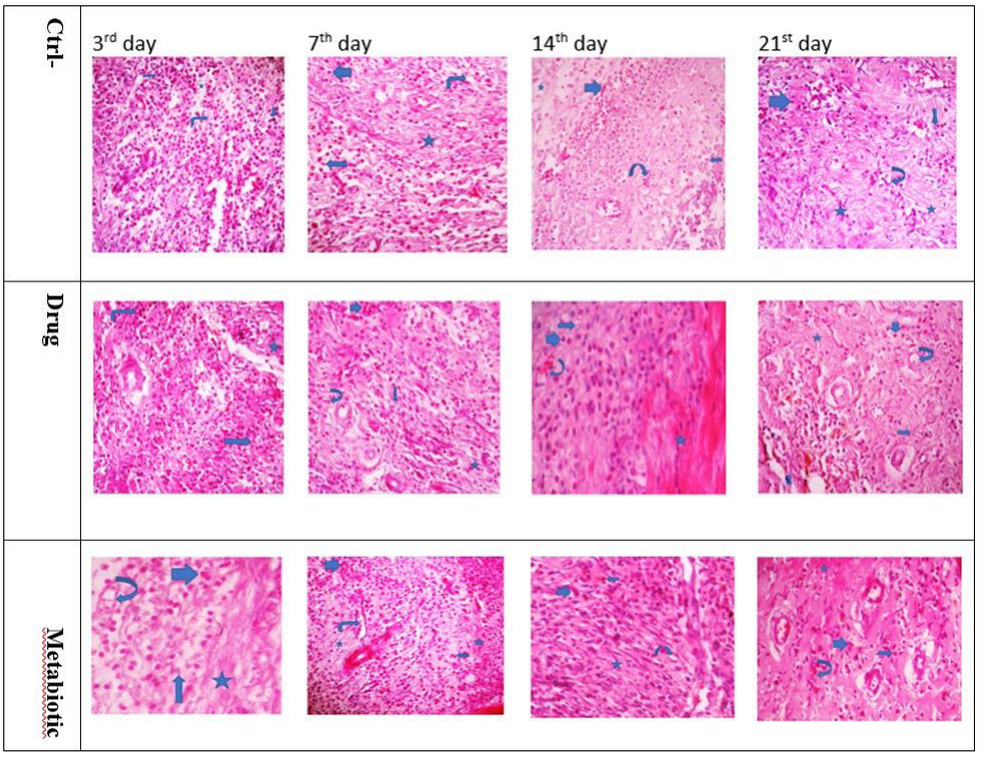

Fig. 1. The microscopic view of a peptic ulcer healing in Ctrl-, drug and metabiotic groups on day 3, 7, 14 and 21 post wounding (Hematoxylin-eosin staining, magnification: × 250). Thick arrow indicates the infiltration of polymorphonuclear neutrophils; Narrow arrow indicates the infiltration of mononuclear inflammatory cells; Star indicates the presence of fibroblasts; and curved arrow indicates the level of angiogenesis.

Table 3. The SOD, GPX, PDGF, and EGF genes expression in group on day 7 and 14 PW.

Gene	Group	Fold increase in gene expression Mean ± SEM	
		Day 7	Day 14
PDGF	Ctrl-	1.26 ± 0.06^{b}	1.82 ± 0.06^{b}
	Drug	2.93 ± 0.12^{a}	3.80 ± 0.11^{a}
	Metabiotic	2.70 ± 0.14^{a}	3.91 ± 0.09^{a}
IL-8	Ctrl-	1.61 ± 0.15^{c}	2.61 ± 0.19^{a}
	Drug	3.55 ± 0.17^{b}	1.37 ± 0.14^{b}
	Metabiotic	4.58 ± 0.15^{a}	1.71 ± 0.15^{b}
EGF	Ctrl-	1.55 ± 0.05^{c}	1.94 ± 0.11^{b}
	Drug	3.12 ± 0.12^{b}	3.82 ± 0.15^{a}
	Metabiotic	4.96 ± 0.14^{a}	3.97 ± 0.23^{a}
GPx	Ctrl-	1.30 ± 0.10^{c}	1.72 ± 0.11^{b}
	Drug	2.63 ± 0.13^{a}	3.15 ± 0.15^{a}
	Metabiotic	2.51 ± 0.37^{a}	3.12 ± 0.10^{a}
SOD	Ctrl-	1.59 ± 0.08^{b}	1.54 ± 0.11^{b}
	Drug	2.53 ± 0.05^{a}	3.25 ± 0.09^{a}
	Metabiotic	1.77 ± 0.15^{a}	1.22 ± 0.11^{b}

Means with the same letter in a column are not significantly different from each other.

thing was that in the Ctrl- group, the expression level of PDGF did not differ significantly from the 7th to the 14th day. While in the drug and metabiotic groups, these levels were significantly higher on the 14th day. This indicates a faster start of the proliferation phase in these two groups.

The results of this research showed that on 7-day PW, the expression level of this interleukin was higher in the treated groups than in the Ctrl- group (P value < 0.001). Interestingly, the use of probiotics induced more IL-8 gene expression compared to the drug group. On the 14-day PW, the expression of this pro-inflammatory interleukin in the treated groups had decreased significantly compared to the Ctrl- group. This indicated the resolution of inflammation in these two groups and there was no significant difference between them (P value=0.362). Comparison of the expression kinetics of IL-8 in the ctrl- group showed a significant increase from day 7 to day 14 PW. In other words, the phase of inflammation and the widespread calling of PMNs continued until the 14th day. While in the other two groups, the resolution of inflammation was confirmed with a decrease in the expression of this pro-inflammatory cytokine from day 7 to day 14 PW.

Our results showed that the administration of the drug and metabiotic significantly increased GPX gene expression on days 7 and 14 (PW). There was no significant difference in the expression of this gene in these two treated groups. Surprisingly, the expression of SOD in the drug group was higher than the Ctrl- and metabiotic group in the mentioned days. No significant difference was observed in the expression of this gene in metabiotic and negative control groups (Table 3).

DISCUSSION

Wound healing is a complex cellular system including overlapping phases of homeostasis, inflammation, proliferation, and remodeling. In the homeostasis phase, a set of inactive zymogens become active serine proteases and lead to the activation of platelets and the formation of a fibrin clot. Fetal platelets produce growth factors such as PDGF and inflammatory mediators such as IL-8. PDGF is involved in the chemotaxis, proliferation, and expression of new genes in monocyte-macrophage and fibroblasts, while IL-8 is a very potent chemokine in neutrophil recruitment. When neutrophils and monocytes enter the wound site, the inflammatory phase begins. This phase is characterized by the production of pro-inflammatory interleukins, ROS, and proteolytic enzymes such as matrix metalloproteinase (MMP). ROS has a very important role in physiological processes and controls different intracellular signaling pathways. It has been proven that appropriate levels of ROS control various stages of wound healing, including blood coagulation, cell infiltration and proliferation, angiogenesis, and re-epithelialization. Besides, ROS increases the expression of cytokines such as EGF, KGF, and TGF-alpha and thus accelerate wound closure (36-39).

It is proved that some enzymes involved in signaling pathways, such as phosphotyrosine phosphatase, have sulfhydryl residues in their catalytic center. So, they are very sensitive to oxidative changes and are deactivated by oxidation. Therefore, the increase in ROS due to the imbalance in oxidative homeostasis creates a signaling network that leads to impaired wound healing (40, 41).

Today, the use of antioxidant compounds for wound treatment has received much attention. Recently, the safety of synthetic antioxidants has been questioned due to liver damage and carcinogenicity (42, 43). So, finding safer and natural antioxidants has attracted a lot of attention. Probiotic bacteria and their produced metabolites are one of the best options that, in addition to improving health, also have antioxidant effects (21, 44).

Our results showed that treatment with metabiotic extracted from *B. bifidum* ATCC 29521 increases the expression of PDGF. Degranulated platelets release a large amount of PDGF at the wound site, which causes mitogenicity and chemotaxis of neutrophils, macrophages, and fibroblasts. PDGF also causes the conversion of fibroblast to myofibroblast, and stimulates ECM production, angiogenesis and re-epithelization. The increase in PDGF expression due to treatment with metabiotic stimulated the infiltrations of neutrophils and monocyte-macrophages in the wound site. As expected, with an increase in the number of macrophages, an increase in IL-8 gene expression was also observed. This interleukin can bind to the surface receptors of neutrophils and causes their active attraction to the wound site (45-47).

Neutrophils in the wound site produce IL-8, which leads to a pro-inflammatory feedback loop (48). In other words, the use of metabiotic led to a faster and more intense inflammatory phase. The increase in the number of these phagocytic cells is associated with the removal of damaged cells and pathogens through the damage-associated molecular pattern (DAMP) and pathogen-associated molecular pattern (PAMP) receptors, respectively (49). But it should be noted that the intensification of the inflammation phase is inevitably accompanied by an increase in ROS levels at the wound site, which, if not controlled, can lead to chronic wounds. Surprisingly, the used metabiotic was able to modulate the antioxidant system. The expression level of SOD and GPx genes increased by using the metabiotic during the inflammatory phase. The antioxidant ability of probiotics has been proven by several investigations (50). Unfortunately, the mechanism of this antioxidant activity is not known precisely.

But it seems that the metabolites produced by probiotics bacteria play an important role in their antioxidant activity. It has been suggested that physiological chelators present in metabiotic can prevent the catalysis of the oxidation process by binding to metal ions such as Fe^{+2} and Cu^{+2} (51, 52). Several studies have shown that short-chain fatty acids, especially butyrate, can activate cellular antioxidant mechanisms and

suppress pro-inflammatory mediators.

Previous studies showed that metabiotic extracted from *B. bifidum* ATCC 29521 contain high amounts of butyrate, acetate, and citrate. Butyrate reduces the secretion of pro-inflammatory cytokines and acetate reduces inflammatory signals, as a result of which the amount of IL-6 and TNF-α interleukins decrease (53, 54). The results of this research showed that the used metabiotic increases the expression of SOD and GPx antioxidant genes, which was in line with the research done by Lee and his colleagues (55).

Among other beneficial effects of this metabiotic was an acceleration of re-epithelization and faster wound closure. EGF is the most important growth factor that increases the proliferation of keratinocytes and migration to the wound site, resulting in re-epithelization. The increase in EGF expression was evident on the 7th day after treatment with metabiotic, compared to other groups. This was also seen in the faster closing of the wound completely so that almost after 7 days, the wound created in the stomach was completely closed.

CONCLUSION

Using this metabiotic is a smart step forward for wound healing because it avoids the risks associated with probiotics. The use of whole cells is always associated with the possibility of adverse effects in immune-compromised hosts. The results of this research showed that metabiotic extracted from *B. bifidum* accelerate the healing process by stimulating and increasing the antioxidant capacity and modulating the inflammatory phase.

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REFERENCES

1. Xie X, Ren K, Zhou Z, Dang C, Zhang H. The global, regional and national burden of peptic ulcer disease from 1990 to 2019: a population-based study. *BMC Gastroenterol* 2022; 22: 58.
2. Lanas A, Dumonceau J-M, Hunt RH, Fujishiro M, Scheiman JM, Gralnek IM, et al. Non-variceal upper gastrointestinal bleeding. *Nat Rev Dis Primers* 2018; 4: 18020.
3. Sumbul S, Ahmad MA, Mohd A, Mohd A. Role of phenolic compounds in peptic ulcer: An overview. *J Pharm Bioallied Sci* 2011; 3: 361-367.
4. Nagasue T, Nakamura S, Kochi S, Kurahara K, Yaita H, Kawasaki K, et al. Time trends of the impact of *Helicobacter pylori* infection and nonsteroidal anti-inflammatory drugs on peptic ulcer bleeding in Japanese patients. *Digestion* 2015; 91: 37-41.
5. Nagata N, Niikura R, Sekine K, Sakurai T, Shimbo T, Kishida Y, et al. Risk of peptic ulcer bleeding associated with H elicobacter pylori infection, nonsteroidal anti-inflammatory drugs, low-dose aspirin, and antihypertensive drugs: A case-control study. *J Gastroenterol Hepatol* 2015; 30: 292-298.
6. Robinson K, Letley DP, Kaneko K. The human stomach in health and disease: infection strategies by *Helicobacter pylori*. *Curr Top Microbiol Immunol* 2017; 400: 1-26.
7. Mohseni S, Bayani M, Bahmani F, Tajabadi-Ebrahimi M, Bayani MA, Jafari P, et al. The beneficial effects of probiotic administration on wound healing and metabolic status in patients with diabetic foot ulcer: a randomized, double-blind, placebo-controlled trial. *Diabetes Metab Res Rev* 2018; 34: 10.1002/dmrr.2970.
8. Long AT, Kenne E, Jung R, Fuchs TA, Renné T. Contact system revisited: an interface between inflammation, coagulation, and innate immunity. *J Thromb Haemost* 2016; 14: 427-437.
9. Smith SA, Travers RJ, Morrissey JH. How it all starts: Initiation of the clotting cascade. *Crit Rev Biochem Mol Biol* 2015; 50: 326-336.
10. Tarnawski AS. Cellular and molecular mechanisms of gastrointestinal ulcer healing. *Dig Dis Sci* 2005; 50 Suppl 1: S24-33.
11. Jamaran S, Jafari P, Marjani A, Akbari N, Feizabad MM. Novel wound dressing based on postbiotic/chitosan film accelerates cutaneous wound healing. *Jundishapur J Microbiol* 2021; 14(12): e120806.
12. Auf Dem Keller U, Kümin A, Braun S, Werner S. Reactive oxygen species and their detoxification in healing skin wounds. *J Investig Dermatol Symp Proc* 2006; 11: 106-111.
13. Schieber M, Chandel NS. ROS function in redox signaling and oxidative stress. *Curr Biol* 2014; 24: R453-62.
14. Zhu G, Wang Q, Lu S, Niu Y. Hydrogen peroxide: a potential wound therapeutic target. *Med Princ Pract* 2017; 26: 301-308.
15. Sies H, Jones DP. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nat Rev*

Mol Cell Biol 2020; 21: 363-383.
16. Mirończuk-Chodakowska I, Witkowska AM, Zujko ME. Endogenous non-enzymatic antioxidants in the human body. *Adv Med Sci* 2018; 63: 68-78.
17. Belinskaia DA, Voronina PA, Shmurak VI, Vovk MA, Batalova AA, Jenkins RO, et al. The universal soldier: Enzymatic and non-enzymatic antioxidant functions of serum albumin. *Antioxidants (Basel)* 2020; 9: 966.
18. Ighodaro OM, Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alexandria J Med* 2018; 54: 287-293.
19. Kurahashi T, Fujii J. Roles of antioxidative enzymes in wound healing. *J Dev Biol* 2015; 3: 57-70.
20. Nafiu AB, Rahman MT. Anti-inflammatory and antioxidant properties of unripe papaya extract in an excision wound model. *Pharm Biol* 2015; 53: 662-671.
21. Wang Y, Wu Y, Wang Y, Xu H, Mei X, Yu D, et al. Antioxidant properties of probiotic bacteria. *Nutrients* 2017; 9: 521.
22. Libera J, Latoch A, Wójciak KM. Utilization of grape seed extract as a natural antioxidant in the technology of meat products inoculated with a probiotic strain of LAB. *Foods* 2020; 9: 103.
23. Feng T, Wang J. Oxidative stress tolerance and antioxidant capacity of lactic acid bacteria as probiotic: A systematic review. *Gut Microbes* 2020; 12: 1801944.
24. Kim H, Kim J-S, Kim YG, Jeong Y, Kim J-E, Paek N-S, et al. Antioxidant and probiotic properties of Lactobacilli and Bifidobacteria of human origins. *Biotechnol Bioprocess Eng* 2020; 25: 421-430.
25. Happel AU, Barnabas SL, Froissart R, Passmore JS. Weighing in on the risks and benefits of probiotic use in HIV-infected and immunocompromised populations. *Benef Microbes* 2018; 9: 239-246.
26. Haghighat L, Crum-Cianflone NF. The potential risks of probiotics among HIV-infected persons: Bacteraemia due to Lactobacillus acidophilus and review of the literature. *Int J STD AIDS* 2016; 27: 1223-1230.
27. Ferrario C, Duranti S, Milani C, Mancabelli L, Lugli GA, Turroni F, et al. Exploring amino acid auxotrophy in bifidobacterium bifidum PRL2010. *Front Microbiol* 2015; 6: 1331.
28. Guzmán-Gómez O, García-Rodríguez RV, Quevedo-Corona L, Pérez-Pastén-Borja R, Rivero-Ramírez NL, Ríos-Castro E, et al. Amelioration of ethanol-induced gastric ulcers in rats pretreated with phycobiliproteins of Arthrospira (Spirulina) Maxima. *Nutrients* 2018; 10: 763.
29. sowjanya A, Pal A. Drug-Drug-Dietary interactions in pharmacotherapy of GIT medication: A review. *Int J Res Pharm Sci* 2020; 11: 2903-2909.
30. Subudhi BB, Sahoo SP, Sahu PK. Updates in Drug Development strategies against peptic ulcer. *J Gastrointest Dig Syst* 2016; 6: 10.4172/2161-069X.1000398.
31. Wellington D, Mikaelian I, Singer L. Comparison of ketamine–xylazine and ketamine–dexmedetomidine anesthesia and intraperitoneal tolerance in rats. *J Am Assoc Lab Anim Sci* 2013; 52: 481-487.
32. Beeton C, Garcia A, Chandy KG. Drawing blood from rats through the saphenous vein and by cardiac puncture. *J Vis Exp* 2007; (7): 266.
33. El-Ela FIA, Farghali AA, Mahmoud RK, Mohamed NA, Moaty SAA. New approach in ulcer prevention and wound healing treatment using doxycycline and amoxicillin/LDH Nanocomposites. *Sci Rep* 2019; 9: 6418.
34. Feldman AT, Wolfe D. Tissue processing and hematoxylin and eosin staining. *Methods Mol Biol* 2014; 1180: 31-43.
35. Fischer AH, Jacobson KA, Rose J, Zeller R. Hematoxylin and eosin staining of tissue and cell sections. *CSH Protoc* 2008; 2008: pdb.prot4986.
36. Gonzalez AC, Costa TF, Andrade ZA, Medrado AR. Wound healing-A literature review. *An Bras Dermatol* 2016; 91; 614-620.
37. Rodriguez PG, Felix FN, Woodley DT, Shim EK. The role of oxygen in wound healing: a review of the literature. *Dermatol Surg* 2008; 34: 1159-1169.
38. Kimmel HM, Grant A, Ditata J. The Presence of Oxygen in Wound Healing. *Wounds* 2016; 28: 264-270.
39. Velnar T, Bailey T, Smrkolj V. The wound healing process: an overview of the cellular and molecular mechanisms. *J Int Med Res* 2009; 37: 1528-1542.
40. Dunnill C, Patton T, Brennan J, Barrett J, Dryden M, Cooke J, et al. Reactive oxygen species (ROS) and wound healing: the functional role of ROS and emerging ROS-modulating technologies for augmentation of the healing process. *Int Wound J* 2017; 14: 89-96.
41. Nouvong A, Ambrus AM, Zhang ER, Hultman L, Coller HA. Reactive oxygen species and bacterial biofilms in diabetic wound healing. *Physiol Genomics* 2016; 48: 889-896.
42. Xu Z, Han S, Gu Z, Wu J. Advances and impact of antioxidant hydrogel in chronic wound healing. *Adv Healthc Mater* 2020; 9(5): e1901502.
43. Li Z, Zhang J, Fu Y, Yang L, Zhu F, Liu X, et al. Antioxidant shape amphiphiles for accelerated wound healing. *J Mater Chem B* 2020; 8: 7018-7023.
44. Amaretti A, di Nunzio M, Pompei A, Raimondi S, Rossi M, Bordoni A. Antioxidant properties of potentially probiotic bacteria: *in vitro* and *in vivo* activities. *Appl Microbiol Biotechnol* 2013; 97: 809-817.
45. Gentile P, Garcovich S. Systematic review: adipose-derived mesenchymal stem cells, platelet-rich plasma and biomaterials as new regenerative strategies in chronic skin wounds and soft tissue defects. *Int J Mol Sci* 2021;

22: 1538.
46. Finnson KW, Arany PR, Philip A. Transforming growth factor beta signaling in cutaneous wound healing: lessons learned from animal studies. *Adv Wound Care (New Rochelle)* 2013; 2: 225-237.
47. Cakir SN, Whitehead KM, Hendricks HKL, de Castro Brás LE. Novel techniques targeting fibroblasts after ischemic heart injury. *Cells* 2022; 11: 402.
48. Ellis S, Lin EJ, Tartar D. Immunology of wound healing. *Curr Dermatol Rep* 2018; 7: 350-358.
49. D'Arpa P, Leung KP. Toll-like receptor signaling in burn wound healing and scarring. *Adv Wound Care (New Rochelle)* 2017; 6: 330-343.
50. Shen Q, Shang N, Li P. *In vitro* and *in vivo* antioxidant activity of Bifidobacterium animalis 01 isolated from centenarians. *Curr Microbiol* 2011; 62: 1097-1103.
51. Lee J, Hwang K-T, Chung M-Y, Cho D-H, Park C-S. Resistance of *Lactobacillus casei* KCTC 3260 to reactive oxygen species (ROS): role for a metal ion chelating effect. *J Food Sci* 2005; 70: m388-m391.
52. Ahire JJ, Mokashe NU, Patil HJ, Chaudhari BL. Antioxidative potential of folate producing probiotic Lactobacillus helveticus CD6. *J Food Sci Technol* 2013; 50: 26-34.
53. Silva YP, Bernardi A, Frozza RL. The role of short-chain fatty acids from gut microbiota in gut-brain communication. *Front Endocrinol (Lausanne)* 2020; 11: 25.
54. Kim S, Park S, Choi TG, Kim SS. Role of short chain Fatty acids in epilepsy and potential benefits of probiotics and prebiotics: targeting "Health" of Epileptic Patients. *Nutrients* 2022; 14: 2982.
55. Lee JY, Kang C-H. Probiotics Alleviate Oxidative Stress in H2O2-exposed Hepatocytes and t-BHP-Induced C57BL/6 Mice. *Microorganisms* 2022; 10: 234.