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The Impact of Oral Administration of Systemic Probiotics "*Lactobacillus rhamnosus*" on IL-1β and IL-10 Levels and Wound Healing of Oral Mucosa in Rabbits

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Abstract

THE LACK of effective treatment for oral wounds has motivated researchers to explore alternative treatments to improve intraoral wound healing. **Aims**: This study investigates the beneficial role of probiotics *Lactobacillus rhamnosus* in oral wounds. **Material and Method:** Twenty-five rabbits were included and categorized into three groups; The control negative group (five rabbits) who had no surgery and no treatment. The control positive group (ten rabbits), was subdivided equally into two subgroups (a and b) they had an incision of 1cm in their buccal mucosa but had not received any treatment. The third group (ten rabbits), the treatment group, was also subdivided into (a and b) and received probiotic drops of *Lactobacillus rhamnosus* to be swallowed once daily starting from day 1 till the day of euthanizing. The rabbits from group I and subgroups IIa and IIIa were sacrificed on the third day. All rabbits of subgroups IIb and IIIb were euthanized on the seventh day. Serum samples were collected and their mucosal tissue at wound sites was removed for histological analysis. **Results:** The serum levels of proinflammatory Interluekin-1β and anti-inflammatory Interluekin-10 in the third-day groups presented a significant difference, while in the seventh-day groups, the results of the treated group showed a higher level of IL-10 than IL-1B indicating less inflammation. In microscopic inspection, the untreated group revealed more inflammatory signs on day 3 than the treated group. On day 7, the treated group presented very good signs of healing as compared to the control-positive group. **Conclusion:** Systemic probiotic *Lactobacillus rhamnosus* enhances the wound healing of oral mucosa.

Keywords: Probiotics, Oral mucosa, Wound healing, IL-10, Il-1β, Rabbits.

Introduction

Probiotics are alive microorganisms that have useful effects on health and can control pathogenic microbes. The most common probiotics are lactic acid bacteria including Bifidobacter species and Lactobacillus [1]. Although important developments in the pharmacological field, there are currently few medications available that could accelerate the healing process of wounds. One kind of replacement treatment to restore microorganisms is bacterial therapy.

Probiotic bacterial consumption is one approach to it. After ingestion, harmful bacteria face competition from friendly bacteria in fermented food products, which limits their growth. Probiotic bacteria can create antimicrobial compounds or destroy toxins in addition to competing with pathogenic bacteria for resources and adhering to the environment. They can also stop pathogenic bacteria from adhering to cells and invading the environment. Additionally, they work well to control the systemic and local immune systems [2].

The World Health Organization (WHO) described probiotics as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" [3]. Several probiotic bacteria are part of the microbiota of the intestine that may be added to the diet to enhance the health of the gut by preserving the gastrointestinal microbial equilibrium [4]. *Lactobacillus rhamnosus GG* (LGG)

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is one of the most favourable strains of biotherapeutic bacteria since it can grow rapidly, adhering capability, and resist bile acids to stay for a more extended time inside the gut and apply stronger effects as compared to other bacteria, including protecting the gut lining [5].

The complicated microbial ecosystem of the oral cavity keeps a tight connection between the human body and oral microbiota. Many studies suggested that probiotics have valuable oral health benefits as well as on the wound healing of oral mucosa if administered via local or systemic routes, especially after oral surgeries following removal of cancer, cleft palate surgeries, ulcers, or implants.

Hemostasis, inflammation, proliferation, and re-modeling are the four successive phases of wound healing, each of which involves a complex interaction of different cell types [6].

During the inflammatory phase, proliferating cells, growth factors, and cytokines aggregate within the area of damage. Fibroblasts then generate fibronectin and collagen as part of the development of the new extracellular matrix. After that, myofibroblasts constrict the wound margins as epithelial cells migrate over the wound bed. Finally, the remodeling phase enables granulation tissue to evolve into matured connective tissue or scar. Any disruption in the healing process may result in imperfect or delayed healing [7].

The pro-inflammatory cytokine IL-1β has been linked to autoimmune diseases, pain, and inflammation. It is essential for the host's defense mechanisms against damage and infection. Of the members of the IL-1 family, it is also the most well-studied and best characterized. It is produced and secreted by numerous cell types like macrophages and monocytes [7]. While many different cell types produce interleukin -10 (IL-10), a key immunoregulatory cytokine. It appears that its primary biological role is to control the development and proliferation of various immune cells as well as the inhibition and cessation of inflammatory responses [8].

It has been proposed that the health-promoting qualities of probiotics vary depending on the strain. Since probiotics may control cytokine synthesis and trigger antimicrobial immune responses, the strain's identity and properties are crucial. As an example, certain probiotics can produce interleukin (IL)-12, which stimulates natural killer (NK) cells and enhances the secretion of interferon (IFN). But they also promote the development of more IL-10, which in turn suppresses the inflammatory response and produces antibodies, thereby balancing and promoting healing [9].

This study focused on the effect of orally administered *Lactobacillus rhamnosus GG* (LGG) as systemic probiotics on microscopic changes and serum levels of both IL-1 β and IL-10 during oral mucosal wound healing in rabbits.

Material and Methods

Ethical approval

The Research Ethics Committee and the Scientific Committee at the Department of Basic Dental Sciences, College of Dentistry, University of Mosul approved this study. Approval No.: UoM-Dent.23/16.

Animals

Twenty-five local male rabbits, weighing 1-1.5 kg and ranging in age from 12 to 18 months were used in this study. They were housed individually in cages with a specific ambient temperature of 24±2°C, a light-dark cycle of 12 hours, and free access to water and regular food [10].

Experiment

The surgical procedure involved an intramuscular injection of xylazine hydrochloride, a sedative and muscle relaxant, and ketamine hydrochloride, an anesthetic and analgesic, at a dose of 5, 50 mg/Kg respectively into the thigh muscle to anesthetize the animals [11]. The sedated rabbit was then placed on the surgical table. With forceps and surgical blade no. 15, a horizontal incision of 1 cm was created in the animal buccal mucosa. The twenty-five rabbits were divided into three main groups; The first group I (5 rabbits), was named the control negative group, they had no incision and received no treatment. The second group II (10 rabbits), named the control positive group, split into two subgroups (a and b) of five animals each, they had an incisional cut of 1cm in their buccal mucosa but none of them had received any drug throughout the experiment. The Treatment Group III (ten rabbits) was also subdivided equally into a and b subgroups, the same mucosal incision was made and they received daily *Lactobacillus rhamnosus* probiotic Colic Go Drops (Vitron Farma Company®, Turkey), by oral route, 5 oral drops (0.25 ml)/kg from the first day postoperatively till the day of sacrifice.

According to the day of the sacrifice, all rabbits from group I, five rabbits from group IIa, and five rabbits from group IIIa were euthanized on the third day, while the five animals from group IIb and five from group IIIb were euthanized on the seventh day. Five milliliters of blood were collected from the jugular vein of rabbits, put into tubes, and left for thirty minutes at room temperature [12].

After being separated, the serum was moved to an Eppendorf tube and kept cold (-20 ˚C) until it was time for analysis. with rabbit interleukin-1β and

interleukin-10 ELISA kits (Bioassay Technology Laboratory) BT LAB Cat.No E0066Rb and Cat.No E0004Rb respectively. Also, the wounded area was surgically removed with surrounding tissue by scissors, washed with normal saline, and kept for histological analysis in 10% formalin. The histological evaluation was implied by a histopathologist.

Statistical analysis

The results were organized in tables and IBM SPSS Statistics® software, v.25 was used to analyze the data. The results were presented by mean values ± standard deviations. Since the data did not follow the normal distribution according to the Shapiro-Wilk test (P values were lower than 0.05), they were statistically analyzed by nonparametric tests. To compare between two groups, the Mann-Whitney test was used. The comparisons among all groups were done by the Kruskal-Wallis test. Statistical significance was indicated by P values less than 0.05.

Results and Discussion

Serum IL-10 results

Table (4) demonstrates the significant differences of (0.002) among all study groups when compared with the Kruskal-Wallis test regarding this biomarker.

Looking at Table (6), the mean concentration of the control negative group was 486 ±57 ng/l but the control positive group showed a significant reduction to 417±129 ng/l on day 3 but increased gradually till day 7 reaching 738±22 ng/l. On the other hand, the treated group showed a lower concentration of this parameter on 3PO at 337±39 than the control positive, however, it revealed a good rise to 529±9 ng/L at 7PO and reached a level near that of the control negative group that could be considered as a normal state as illustrated in Figure (2).

Histopathological results

Histopathological sections were prepared from each mucosal specimen. Each slide was examined under a light microscope by a well-experienced histopathologist who read inflammatory cell infiltration (ICI), re-epithelialization (RE), and Granulation tissue (GT) scoring. The means of this reading were regarded as a final score.

The microscopic appearance of the healthy buccal mucosa displays a thick non-keratinized stratified squamous epithelium. The lamina propria have dense fibrous connective tissue containing collagen and some elastic fibers, long slender papillae, and a rich vascular supply [13]. The mucosa is tightly attached to the beneath muscle by dense collagenous connective tissue with minor salivary glands and fat as shown in Figure (3).

Microscopic inspection of the oral mucosal tissue taken from the control positive group 3PO, revealed a wound site with inflammatory exudate, containing highly inflammatory cell infiltration, and fibrin deposition, destruction of the epithelium layer of mucosa with slight re-epithelialization (Figure 4). Whereas the 7PO tissue of the same group presented inflammatory exudate, containing highly inflammatory cell infiltration, fibrin deposition, re-epithelialization, and few granulation tissues (Figure 5). In contrast, the histopathological examination of the treated group's mucosal wounds three postsurgical days showed a wound site with inflammatory exudate containing moderate inflammatory cell infiltration, fibrin deposition, destruction of epithelium layer of mucosa with re-epithelialization, and well granulation tissue (Figure 6), while tissue taken from treatment group at day seven showed wound site without the inflammatory exudate with well-developed re-epithelialization and well granulation tissue with angiogenesis (Figure 7).

Based on the following standards, a histological study was conducted to evaluate the factors influencing the wound-healing process:

(1) Inflammatory Cells Infiltration scoring scale. Score 1: Nil. An absence of inflammatory cells was observed in the area. Score 2: Mild. Few inflammatory cells are seen, less than half of the area. Score 3: Moderate. Inflammatory cells might be observed in more than half of the area. Score 4: Severe. Inflammatory cells are seen in abundant quantity, more than three-quarters of the area.

(2) Granulation tissue formation scoring criteria: Score 1: no granulation tissue formed. Score 2: The amount of granulation tissue in the gap is small.

Serum IL-1 β results

The data in Table (1) displayed substantial changes in IL-1 β levels among all groups as the P value was 0.000 in the Kruskal-Walis test. In Table (2), Mann-Whitney tests were performed between every two independent groups and also gave significant values less than 0.05 indicating changes in concentrations of this parameter.

According to Table (3) and Figure (1), the IL-1β mean level revealed a gradual increase from 7.3±0.7 ng/L in the control negative going up to 7.8±0.4 ng/L in the 3 postoperative day (3PO) in the control positive group then decreased to 5.1±0.5 ng/L at the end of the study. On the other hand; the treated group showed a significant rise in 3PO to 9.3±0.6 ng/L, then the mean concentration declined to 6.4±0.6 ng/L at 7PO but remained less than the level of the control negative group which could be considered as the normal level of this cytokine at healthy state.

Score 3: The number of granulation tissue formed is moderate. Score 4: The overall granulation tissue formed is profound.

(3) Grading system for assessment of Re-epithelization. Score 0: Re-epithelialization at the edge of the wound. Score 1: Re-epithelialization covering less than half of the wound. Score 2: Re-epithelialization covering more than 1/2 of the wound. Score 3: Re-epithelialization covering all of the wound, irregular thickness. Score 4: Re-epithelialization covering the whole wound, thickness is normal [14]

In this study, the histological mean scores of surgical wounds were compared from the two different groups illustrated in Table (7) and compared in the bar chart in Figure (8). On the 3rd day, the probiotics group showed early signs of healing with fewer inflammatory cells, epithelial cells migrated toward the surface more rapidly, and more granulation tissue than the control group. On the 7th day, the scores for the treatment group showed the absence of inflammatory cells, better re-epithelialization, formation of new blood vessels, and more granulation tissue.

Discussion

The probiotics' beneficial effects have been broadly applied in enhancing health and for the treatment of various infections and diseases in animals. Specifically preventing pathologies, reducing symptoms of irritable bowel syndrome IBS, retardation of the growth of Helicobacter pylori, arresting cancer cells, reducing gut inflammation, and allergic reactions suppressing [4].

The use of probiotics can be topical or systemic. Oral administration enhances the microbiota of the intestine and the absorption of crucial nutrients for wound healing, like minerals, vitamins, and cofactors for the enzymes that control the repair of the oral mucosa [15].

Orally administered by probiotics work by regulating the host microbiota, e.g., by suppressing pathogens, making active metabolites, modulating the activity of the mucosal defense system, triggering cellular pathways within the epithelial cells, modulating the nervous system activity, or changing gene expression of some inflammatory mediators. In addition, any or all of the aforementioned consequences could happen at the same time, which ultimately will stimulate different signal types, triggering several physiological processes in the host that affect oral wound healing [16].

Based on research done on mice, Moriera et al. (2021) explained the possible mechanism of systemic probiotics on remote tissue like the skin, The majority of the health benefits linked to Lactobacillus are attributed to its capacity to generate a wide variety of bioactive metabolites and acids, including carbon dioxide, hydrogen peroxide, bacteriocin, diacetyl, and organic acids (lactic and acetic acids). Nevertheless, little is known about the processes behind the advantageous effects of probiotics when taken orally on distant tissues. The substances that are causing this activation may be lactobacillus structural elements or metabolic products [17].

Lactobacillus rhamnosus GG is a probiotic strain that shows great promise. Due to its fast growth, sticky nature, and resistance to bile, this particular strain of bacteria can outlive other strains in the gut and have a greater impact, including protecting the intestinal lining. It has been discovered that LGG itself protects intestinal epithelial cells and lowers inflammatory indicators like IL-1, interleukin-8 (IL-8), and C-reactive proteins (CRP) [5].

Serum Analysis Discussion:

IL-1β is a pro-inflammatory cytokine that has been implicated in pain, autoimmune conditions, and inflammation. It is necessary for host-defense responses to injury and infection. It is produced by a variety of cell types such as macrophages and monocytes. Numerous physiological processes have been linked to IL-1β. It can control gene expression and cytokine production, influencing angiogenesis, cellular adhesion and migration, and immune response [18].

In this study, IL-1β level was firstly increased mostly in the untreated group than in treated ones throughout the whole experiment, and it was responsible for the inflammatory signs in both groups during the early phase, but at the end of the trial, the high level of IL-1β in the untreated group could be the reason for incomplete healing on the seventh day as compared to its level in the treated group where the low level of pro-inflammatory cytokine is a good indicator of better healing due to the effect of probiotics intake.

An important phase in the healing of wounds is inflammation. However, a significant and protracted inflammatory response often leads to a delayed wound. Strong inflammatory system regulator IL-10 appears to be involved in the development as well as the resolution of general inflammatory responses. Many cell populations produce interleukin-10, an important immunoregulatory cytokine. Its primary biological role appears to be the control of the development and multiplication of various immune cells, including mast cells, B cells, T cells, natural killer cells, antigen-presenting cells, and granulocytes, as well as the inhibition and termination of inflammatory responses. Nonetheless, recent findings indicate that IL-10 also suppresses

the synthesis of IL-1β and modulates immunostimulatory characteristics, aiding in the removal of both infectious and non-infectious particles while reducing inflammation [19].

As a whole, the potential of IL-10 appears to be rather complex, and it may be oversimplifying to simply think of IL-10 as an immunosuppressive and anti-inflammatory drug (as was done in the past). It may be more appropriate to see IL-10 as immunoregulatory rather than immunosuppressive.

It, directly and indirectly, inhibits inflammation-associated immune response (Th1, proinflammatory cytokine production by macrophages, Th2 modulation), but it enhances functions of Th2-related immunity and natural immunity (NK cell activity and noninflammatory elimination of particles and microbes by activating phagocytosis) [20].

In this study, the expression level of the anti-inflammatory cytokine IL-10 in the control positive group on 3rd day was less than that of the control negative group but more than the treated group which showed more depression possibly due to the overwhelmed expression of the pro-inflammatory cytokine IL-1 β at this stage of inflammatory process on 3PO. In comparison to the 7th day, levels almost doubled in the untreated and treated group, explaining the activation of the anti-inflammatory process at a late stage of wound healing.

Histopathological discussion:

Several biological processes are triggered right away after tissue damage to rebuild the damaged tissue. In addition to endothelial cells, keratinocytes, and fibroblasts, the immune system's monocytes, neutrophils, lymphocytes, and dendritic cells are among the cells involved in wound healing [7].

In response to chemokines at the point of damage, the wound experiences rapid inflammatory infiltration after the initial hemostasis phase. After an injury, the inflammatory response peaks 24 to 48 hours later and can continue for up to a week. This supports the results of the serum analysis process where there are fewer blood vessels, fewer resident cytokines, and faster fibroblast growth at the wound bed during the early inflammatory phase. However, early inflammation encourages immune cell-mediated elimination of pathogens and debris to transform the matrix into new tissue. First to arrive at the wound site, neutrophils debride damaged extracellular matrix (ECM) components and release proteases such as matrix metalloproteinase (MMP) [21].

Neutrophils then trigger a series of growth factors and cytokine production in the initial inflammatory phase to draw in other immune cells, such as monocytes, which aid in the start of re-epithelialization. Neutrophils leave the wound bed by phagocytosis, apoptosis, and extrusion once it is free of bacteria. Monocytes migrate to the wound and develop into macrophages within 48 to 72 hours following the injury. During the early inflammatory phase, these macrophages preferentially polarize as "pro-inflammatory" M1 macrophages, making them the dominating cell type [22].

Macrophages release a variety of cytokines that facilitate the migration of keratinocytes and fibroblasts to the wound bed, such as interleukin-1, interleukin-6, platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and TGF-b. M2 macrophages assist in lowering previously released "pro-inflammatory" cytokines close to the lesion and increasing endogenous "anti-inflammatory" cytokines. Through "anti-inflammatory" M2 macrophage polarization and continuous release of regenerative cytokines such as interleukin-10, macrophages facilitate proliferative repair during the late inflammatory phase. After infections are cleared by immune cells, increased blood vessel permeability and transudate outflow from capillaries happen, opening a path for the proliferative phase [23].

The probiotic group on the 7PO experienced a faster proliferative phase, characterized by the appearance of fibrosis, in comparison to the controls. This led to a reduced wound tissue area at that time. Probiotic use most likely improved the healing process by encouraging collagen deposition and fibrosis.

Effective wound healing is characterized by the creation of new blood vessels and the restoration of preexisting vascular networks. The process by which new blood vessels proliferate from pre-existing vascular networks to establish microcirculation, support collagen crosslinking, and wound maturation, and restore tissue perfusion is known as “angiogenesis”. Vascular endothelial growth factor (VEGF) is a protein that promotes blood vessel creation and helps with the migration, differentiation, and proliferation of endothelial cells. Singh et al (2017) explained the role of oral treatment with LGG in increasing the levels of the pro-angiogenic VEGF (Vascular Endothelial Growth Factor) cytokine in the wound site and described “Angiogenesis” as an essential element of wound healing that transports nutrients and oxygen to the new tissue for cell metabolism. Both the blood flow and the density of blood vessels in the granulation tissue were enhanced by the oral administration of LGG [24].

Starting from the outside inward, the healing process improves the collagen's structure by reorganizing the fibers. Greater type III collagen

filling was applied to the scar area on the 7PO in the probiotic group, but not in the control group. This led to increased type I collagen production [25].

Granulation tissue, which is made up of fibroblasts, blood vessels, and several leukocyte subtypes such as mast cells, neutrophils, and macrophages, is essential for the healing of wounds. The numerous inflammatory mediators generated following the damage activate and attract these cells to the wound site[26]. In the bar chart in Figure (8) of this study, ICI disappeared gradually in the treated animals by scoring a mean rank of 0 on 7PO, indicating less or no inflammation (i.e. better healing) as compared to the untreated animals at this stage. Another sign of better quality of mucosal healing in the probiotic group was the higher rate of re-epithelialization and granulation tissue formation in the group where LGG was administered daily for a whole week.

Conclusions

The result of this study implies that orally given LGG probiotics to rabbits with oral wounds, resulted in improved healing of mucosal wounds among the treatment group and revealed a significant increase in the level of serum anti-inflammatory IL-10 capacity and a significant decrease of serum pro-inflammatory IL-1β, concluding that using of probiotics might have promising role in treatment of oral wounds and other dental, dermal and medical problems in animals and human.

The use of systemic probiotics after surgery may alter the course of oral wound healing. Probiotics might be used as a complementary to other oral wound management aids to help the oral mucosa recover faster and better after oral surgeries.

A small sample size is one of the research's limitations. However, we believe that systemic probiotic administration has a good impact on the oral mucosa's ability to repair wounds in rabbits and may have a similar effect in people. More investigation is needed to validate these results.

Acknowledgment

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Conflicts of interest

The authors declared no competing interests.

Funding statement

No funding has been provided.

TABLE 1. Comparison of serum IL-1 β among groups.

Test Statistics [a]	IL-1 β
	All Groups
Kruskal-Wallis H	21.720
Df	4
Asymp. Sig.	0.000**

a. Kruskal Wallis Test

**. Highly significant difference.

TABLE 2. Comparison of serum IL-1 β between each two groups.

IL-1 β	Mann-Whitney tests for every 2 independent samples	
Group:	**With group:**	**Sig.**
I	II a	0.009**
	II b	0.009**
	III a	0.009**
	III b	0.028*
II a	II b	0.009**
	III a	0.009**
II b	III b	0.009**
III a	III b	0.009**

*. Significant difference

**. Highly significant difference

TABLE 3. IL-1β levels (mean ± SD) in the serum of study groups.

Descriptive Statistics					
	N	Minimum	Maximum	Mean	Std. Deviation
Control-ve day3	5	6.33	8.40	7.3968	.74849
Control +ve day3	5	7.34	8.36	7.8798	.46463
Control +ve day7	5	4.22	5.64	5.1454	.54629
Treatment day3	5	8.51	9.95	9.3328	.60981
Treatment day7	5	6.01	7.20	6.4058	.46125

TABLE 4. Comparison of serum IL-10 among all groups.

Test Statistics [a]	IL-10
	Groups
Kruskal-Wallis H	17.457
Df	4
Asymp. Sig.	**0.002

a. Kruskal Wallis Test

**. Highly significant difference

Table (5) compares every single group with other independent groups separately using Mann-Whitney tests illustrating the presence of significant difference in IL-10 concentration in compared groups with a P value of (0.009).

TABLE 5. Comparison of serum IL-10 between each two groups.

IL-10	Mann-Whitney tests for every 2 independent samples	
Group:	**With group:**	**Sig.**
I	II a	0.009**
	II b	0.009**
	III a	0.009**
	III b	0.463
II a	II b	0.009**
	III a	0.347
II b	III b	0.009**
III a	III b	0.009**

**. Highly significant difference

TABLE 6. IL-10 levels (mean ± SD) in the serum of study groups.

Descriptive Statistics					
	N	Minimum	Maximum	Mean	Std. Deviation
Control-ve day3	5	456.94	588.78	486.4952	57.44452
Control +ve day3	5	289.43	600.92	417.6182	129.25168
Control +ve day7	5	708.74	762.68	738.2306	22.21647
Treatment day3	5	284.21	379.03	336.9532	39.74737
Treatment day7	5	514.24	537.76	529.5158	9.98167

TABLE 7. Comparing histological scores between treated and untreated groups on the 3rd and 7th day.

Group	Day	ICI	RE	GT
Control+	3PO	3	1	1
Treatment	3PO	2	2	3
Control+	7PO	3	2	2
Treatment	7PO	0	3	3

ICI: Inflammatory Cell Infiltration
RE: Re-epithelialization
GT: Granulation Tissue
PO: Postoperative days

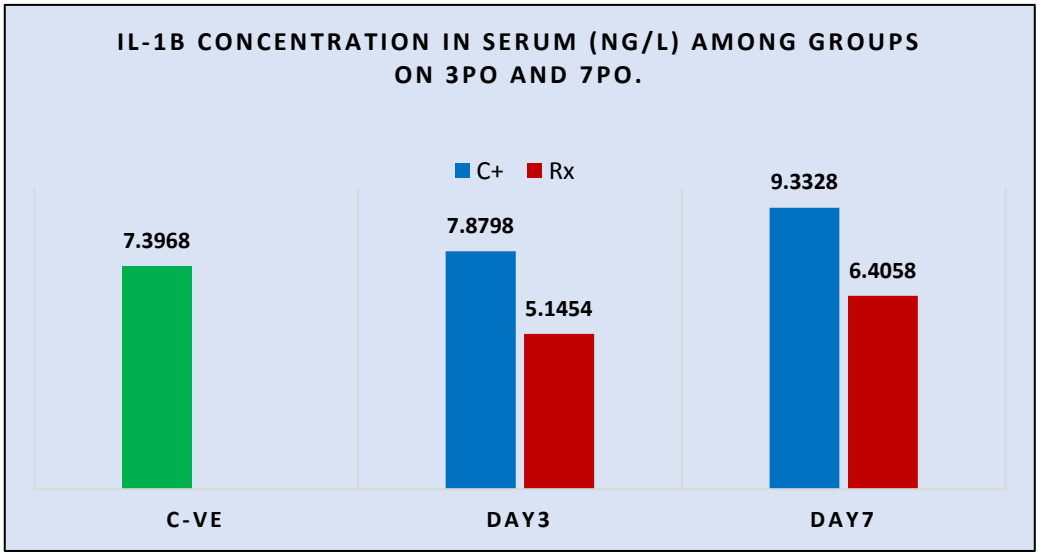

Fig. 1. IL-1 β levels among study groups on days three and seven.

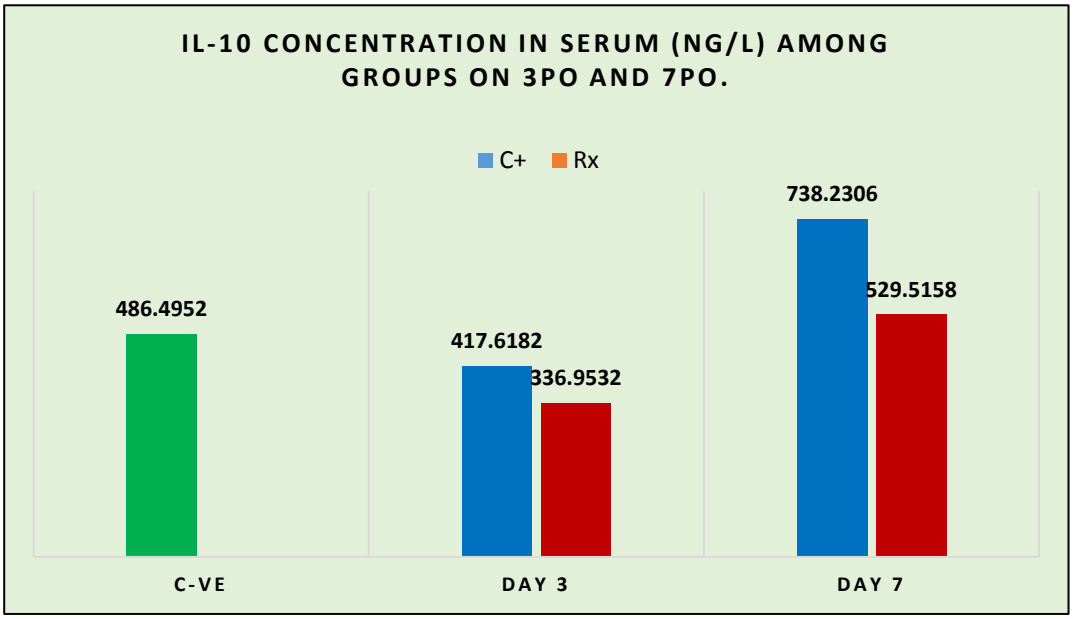

Fig. 2. IL-10 levels among study groups on days three and seven.

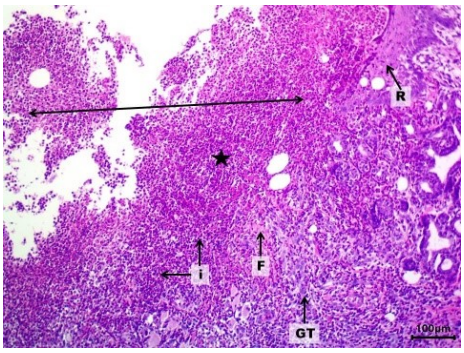

Fig. 3. light micrograph of buccal mucosa of normal rabbit in group I displaying the lamina propria (L) have long slender papillae, very thick non-keratinized stratified squamous (black arrow) of the covering epithelium. The firm attachment of mucosa to the underlying muscle (M) by dense collagenous connective tissue with minor salivary glands (G) and fat (F). H&E.[X-40].

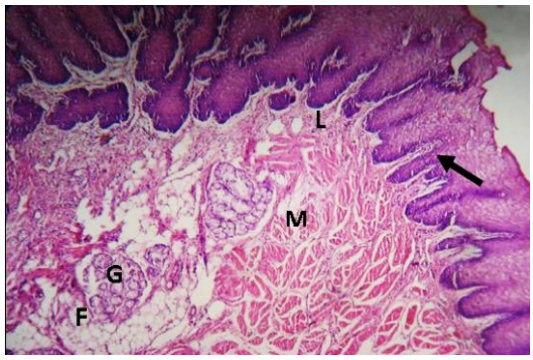

Fig. 4. Histological section of rabbit oral mucosa of control positive (No treatment group) on 3PO showing wound site (↔) with the inflammatory exudate (star), containing highly inflammatory cells infiltration (score 3) (I), and fibrin deposition (F), destruction of epithelium layer of mucosa with re-epithelialization (score 1) (R), and few granulation tissue (score 1) (GT). H&E stain, 100X.

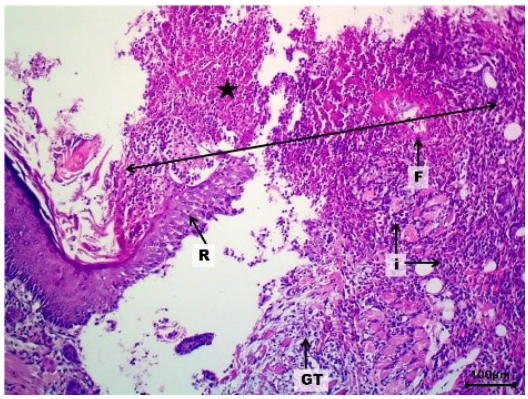

Fig. 5. Histological section of rabbit oral mucosa of control positive (No treatment) group on 7PO revealing wound site (↔) with the inflammatory exudate (star), containing highly inflammatory cells infiltration (score 3) (I), and fibrin deposition (F), re-epithelialization (score 2) (R), and few granulation tissue (score 2) (GT). H&E stain, 40X.

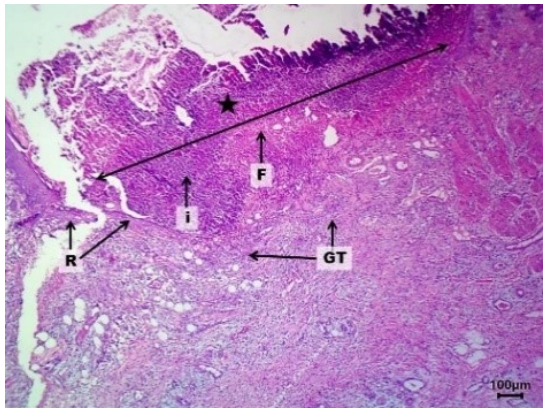

Fig. 6. Histological section of rabbit oral mucosa of the treatment group after on 3PO revealing wound site (↔) with the inflammatory exudate (star), containing moderate inflammatory cells infiltration (score 2) (I), and fibrin deposition (F), destruction of epithelium layer of mucosa with re-epithelialization (score 2) (R), and well granulation tissue (score 3) (GT). H&E stain, 40X.

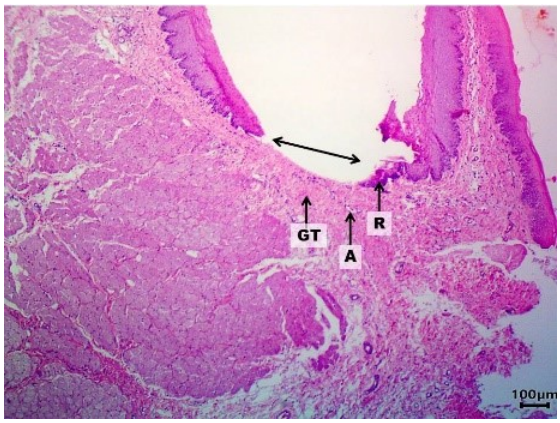

Fig. 7. Histological section of rabbit oral mucosa of the treatment group on 7PO revealing wound site (↔) without the inflammatory exudate (score 0) with well-developed re-epithelialization (score 3) (R), and well granulation tissue (score 3) (GT) with angiogenesis (A). H&E stain, 40X.

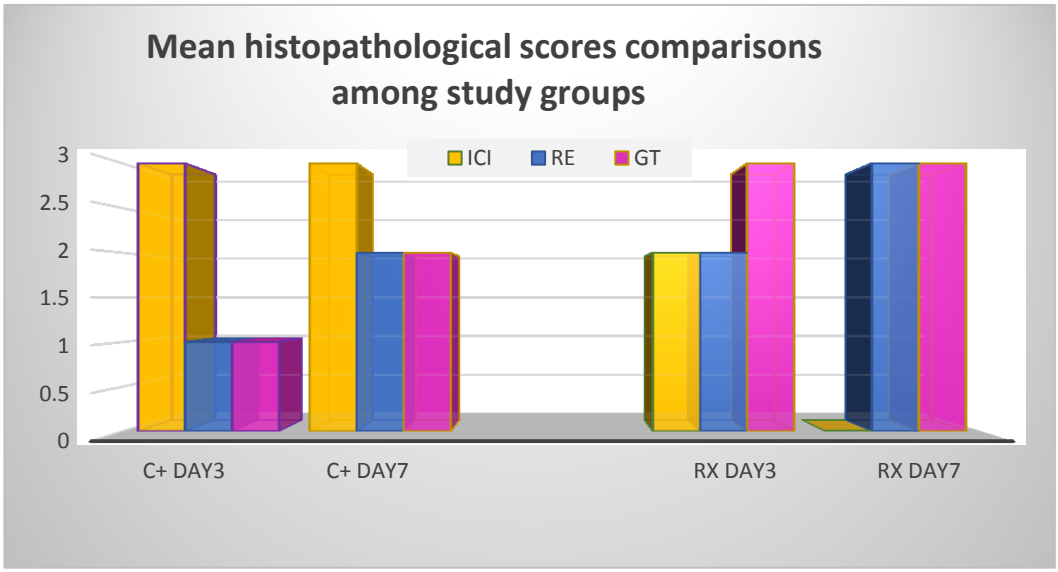

Fig. 8. Comparison of histopathological mean scores between Control and Treatment Groups at day 3 and day 7. (ICI)= Inflammatory Cell Infiltration; (RE)= Re-epithelialization; (GT)= Granulation Tissue; (C+) = Control Positive group; (Rx)= Treatment group.

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تأثير تناول البروبيوتك *Lactobacillus rhamnosus* عن طريق الفم على مستويات (الانترلوكين-10) و (الانترلوكين-1 بيتا) وعلى شفاء جروح بطانة الفم المخاطية في الارانب

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ان غياب العلاج الناجح لمشكلة التئام الجروح في بطانة الفم قد الهم الباحثين للبحث عن بدائل علاجية لتحسين عملية التئام الجروح داخل الفم. تهدف هذه الدراسة في الدور النافع للبكتريا العصوية Lactobacillus rhamnosus في تحسين التئام الجروح الفموية. الدراسة شملت خمسة وعشرون أرنباً، تم تقسيم الارانب المختبرية الى ثلاث مجاميع رئيسية: مجموعة السيطرة السالبة I (خمسة ارانب) الذين لم تجرى عليهم أي عملية جراحية ولم يتم تجريعهم أي دواء. ومجموعة السيطرة الموجبة II (عشرة ارانب)، تم تقسيمهم الى مجموعتين فرعيتين : أ، ب (خمسة ارانب لكل واحدة)، تم عمل جرح في النسيج المخاطي لبطانة الفم قياسه واحد سينتمتر، ولم يتم تجريع هذه المجموعة أي علاج. اما المجموعة الثالثة (عشرة ارانب) والمسماة بمجموعة العلاج، تم تقسيمهم ايضا الى مجموعتين فرعيتين بالتساوي: أ،ب وتم عمل جرح في البطانة المخاطية للفم وتجريعهم البروبايوتك ليتم ابتلاعه الى الجهاز الهضمي وذلك بتقطير خمس قطرات فموية (0.25 مل/كغم) من محلول ال(VITRON) الجاهز والمحتوي على Lactobacillus rhamnosus بجرعات يومية منذ بداية التجربة الى نهايتها. تم قتل الحيوانات من المجاميع (I, IIa, IIIa) بطريقة القتل الرحيم في اليوم الثالث، أما الحيوانات في المجموعتين (IIb, IIIb) فقد ذبحت بالقتل الرحيم في اليوم السابع. تم جمع عينات الدم واستخراج السيرم لتحليلها مختبريا، وتم اقتطاع النسيج المخاطي المحيط بمنطقة الجرح لغرض إجراء التحليل النسيجي. كانت مستويات الانترلوكين-1 بيتا وانترلوكين-10 في مجاميع اليوم الثالث من الدراسة تشير الى وجود فرق معنوي عالي، وكذلك الحال بين مجاميع اليوم السابع والتي أظهرت وجود فرق احصائي ملحوظ في تراكيز هذه المؤشرات المناعية. أظهر الفحص النسيجي للجرح في اليوم الثالث لمجموعة السيطرة الموجبة وجود علامات التهاب عالية اعلى من تلك التي في مجموعة العلاج، بينما في اليوم السابع أظهرت مجموعة العلاج علامات التئام الجرح بشكل جيد جدا مع تكوين الأوعية الدموية مقارنة بالمجاميع الاخرى . يعزز تناول البروبايوتيك عن طريق الفم Lactobacillus rhamnosus من شفاء الجروح في الغشاء المخاطي للفم.