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Investigation of multifunctional potential of *Lactobacillus acidophilus*-DSM20079 in wound infection model

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Abstract: Bacterial colonization and infection remain the major causes of delayed healing following burns. Topical treatment is necessary to reduce the incidence of burn wound infection. However, this treatment produces adverse reactions and side-effects. Lactic acid bacteria (LAB), are very important organisms because, they have no harmful effects, and are available in fermented food. The study was aimed to evaluate the potential applications of *Lactobacillus acidophilus* DSM20079 as the anti-inflammatory and antimicrobial against several pathogenic organisms as well as its effect on wound healing., we performed this exploratory study to establish the effectiveness of bacteriotherapy with topical application of the bacteria *Lactobacillus acidophilus* DSM20079 to provide an alternative method for burn treatment using chloramphenicol as microbicidal agent as a reference drug. These bacteria would compete with other bacteria that are wound pathogens and would modify the wound environment and promote tissue repair. *L. acidophilus*-DSM20079 filtrate and lyophilized cells demonstrated significant antimicrobial properties. The highest antibacterial effects were detected against *Staphylococcus aureus* then *Escherichia coli* and *Micrococcus luteus*, respectively. The in vitro anti-inflammatory action was tested using lipoxygenase enzymatic inhibition assay where *Lactobacillus acidophilus* DSM20079 lyophilized cells solution showed good inhibition of lipoxygenase action with IC_{50} value 13.3±2.1 µg/ml, while IC_{50} =12.7±1.9µg/ml for *Lactobacillus acidophilus* DSM20079 filtrate versus standard dug IC_{50} value of 1.3±0.7 µg/ml. Agar well diffusion assay was also performed to test the antibacterial action against 8 bacterial strains. Moreover, crude metabolites of *Lactobacillus acidophilus* DSM20079 had a significant antimicrobial activity among different bacterial strains. The highest inhibition zone value (20.2±1.8 mm) of metabolites of *Lactobacillus acidophilus* DSM20079 was observed for the *S. aureus* strain.

Keywords: *Lactobacillus acidophilus*, cytokine, Anti-inflammatory.

1.Introduction

Probiotics are living microorganisms that, once given to a recipient in sufficient quantities, have a positive influence on their health and it is suitable for use by humans [1]. Probiotics from the *Lactobacillus* genus are the most often used e.g: *Lactobacillus acidophilus* [2, 3] It is frequently present in yoghurt and many commercially probiotic products. It can produce bacteriocins and organic acids that can stop the development of harmful microbes. Probiotics have been shown to have a variety of consequent effects, including reducing serum cholesterol, antihypertensive effects, the therapeutic advantage in inflammatory bowel ailment, reducing anaphylactic reactions, preventing tooth decay, and

immunomodulatory effects, which are health-promoting effects beyond gut well-being. It is now known that there are distinct changes among diverse probiotic species and strains, and these variations allow for the choice of organisms more logically to cure an ailment. Probiotics have been linked to a variety of new innovative technologies by which they impose their favorable impacts Infections with bacteria are highly prevalent in the environment. When bacterial derivatives such as lipopolysaccharide, which have pathogen-associated molecular patterns (PRRs), enter the host, the host reacts to the infection. An important stage in the development of the innate immune response is the attachment to PRRs on circulating leukocytes. Leukocytes are stimulated by the consequent signaling to generate huge amounts of inflammatory mediators, such as chemokines and cytokines. This inflammatory reaction aids in the removal of microorganisms, but if it is severely raised, it may result in tissue damage or organ damage [4, 5]. The conventional therapy for bacterial infections is antibiotics excessively can lead to antibiotic resistant bacteria [5]. It's crucial to note that bacteria have a defense system, such as the ability to create biofilms, that allow them to avoid antibiotic activity [6]. According to reports, the innate immune system's capacity to eliminate microorganisms is diminished by the bacteriostatic drug chloramphenicol [7] .

A natural healing reaction to tissue injury is wound healing. In order to promote skin resurfacing, reconstruction, and the recovery of the structural integrity of damaged skin, healing entails a sequence of cellular activities. Hemostasis, inflammation, proliferation, and maturation are the four overlapping distinctive phases that make up the orderly process of healing [8]. As a result of the wounds, the physical skin barrier that often keeps microbes out creates new environments for bacterial colonisation, invasion, and acute sepsis. A poor cosmetic outcome or a loss of functionality may follow a prolonged recovery [9]. Numerous bacterial species can cause skin infections. The most common trigger of skin infections is *Staphylococcus aureus*. *Staphylococcus aureus* is a long-term carrier in around 20% of the population [10, 11]. This work was carried out to assess the *Lactobacillus acidophilus's* treatment efficacy versus wound infections rats. The filtrate and lyophilized form of this isolate was also assessed to that of other antibacterial medications used to treat burn wound conditions.

2. Materials and methods

Lactobacillus acidophilus-DSM20079 was purchased from the American Type culture collection (ATCC, USA). This isolate was re-cultured on MRS media, and it was incubated anaerobically for 24 hours at 37°C**.** *Lactobacillus acidophilus* -DSM20079 were cultured in broth medium to obtain filtrate and grown in solid medium then further lyophilized to obtain lyophilized samples. Both *Lactobacillus* filterate as well as lyophilized samples were kept at -20 °C for further testing.

In-vitro antimicrobial Action

Using the Agar-well diffusion technique, the antibacterial activity of *Lactobacillus acidophilus*-DSM20079 was examined against a range of standard bacterial strains. A liquid culture of bacterium with $1x10^8$ cells/mL was used to create a 1% concentration *Lactobacillus acidophilus* solution for susceptibility testing on Muller-Hinton agar, then the resulting inhibition zones were noted [12].

Chloramphenicol was used as standard antibacterial agents (Sigma, USA) for comparison with the tested samples.

In vitro anti-inflammatory assay

Filtrate and lyophilized samples of *Lactobacillus acidophilus*-DSM20079 using LOX enzyme with slight modifications where the Glycine max (type I-B) used to inhibit it in order against the reference chemical (Ibuprofen) to study the anti-inflammatory effect. In 96 well plates, various sample concentrations were combined with 100 µl of soybean LOX solution (1000 U/ml in borate buffer solution, pH 9) and 200 µl of borate buffer to produce a final concentration range of 0.98-125 µg/ml at 25 °C for 15 min. To begin the reaction, 100 µl of linoleic acid was pre-incubated with the sample. Using a microplate reader (BIOTEK; USA), the inhibitory activity was assessed at 250 nm [13].

Animals used

Healthy female Wistar rats weighing 140–180 g were obtained from the Animal house in

Faculty of Science, Al-Azhar University and maintained separately under regular environmental circumstances. They were given pellet rodent meal and water at will, and were given a week to acclimate. The institutional animal ethics committee in the Regional Center for Mycology and Biotechnology gave its approval to the project (RCMB01082021).

Wound infection model

The rats were divided into control and treatment groups (n = 5) for each wound model after being deprived the night before the experiment. Under aseptic conditions and thiopentone anaesthesia, all wounding operations were completed where a full-thickness incisional wound measuring 10 mm in diameter was created in the animal's dorsal part of skin and use an aseptic excisional punch. As a negative control, **Group I** contains 10% sodium lauryl sulphate. **Group II:** a wound procedure was done and a positive control infection of 0.5ml of 10^5 *S. aureus* was done , **Group III:** involved performing the wound and infection using 0.5ml of 10^5 *S. aureus* was done , topical treating it with 1 ml of *Lactobacillus acidophilus*-DSM20079 filtrate which applied twice daily for 21days, and **Group IV** involved performing the wound and infection using 0.5ml of 10^5 *S. aureus*, topically treating it with 1 ml *Lactobacillus acidophilus*-DSM20079 solution of lyophilized cells (0.5 gm of lyophilized cells dissolved in 1 ml deionized sterile water) applied twice daily for 21 days. **Group V:** This group underwent wound and 0.5ml of 10^5 *S. aureus*, and received topical treatment with chloramphenicol (2.8 mg/kg). Animals in each group give individual housing. Photos of the lesions were acquired and applied to detect variations along time points. Also, the body weights were recoded and compared in the same time points [14].

Microbiological Assessment

Swabs (Delta Lab, Spain) were taken at the end of the experiment to gauge the amount of bacteria present in the wounds. Swabs were taken from anesthetized animals to prevent contamination, then plated on mannitol salt agar. The analysis of samples was done using standard microbiological techniques. The number of bacteria was determined by serial dilution in PBS buffer, and the results were expressed as CFU/mL and log10. The cutoff point for an established *S. aureus* cutaneous infection was 10^5 CFU/mL. Furthermore, after sacrificing animals where cervical dislocation used different animals' liver and spleen were gathered, processed, and then cultured on mannitol salt agar [15, 16].

Histopathological Research

After 21 days, clinical healing was evident at the end of the experiment, biopsies were taken by full-thickness excision of the skin. Samples were processed after being fixed in 10% phosphate buffered formalin solution. Tissues were embedded in paraffin, cut into 4 µm sections and stained with hematoxylin and eosin. Furthermore, in order to compare the differences in mast cells in various groups, toluidine blue dye was utilized. Examination and capturing of photos were done and seen under a Nikon microscope (Nikion 40). Nicon camera (Japn Ltd.) was used to capture photos [17] .

Analysis of cytokines

Blood was collected from the rats' eyes were obtained before they were killed, and they were separated in anticoagulated Eppendorf tubes at 6000 rpm for 10 min at 5°C. Then, serum was collected and put into storage at -80°C for analysis. Using kits from Abcam (U.S.A.), we assessed the serum levels of IFN-γ, IL-4, TNF-α, and IL-17 in accord with the manufacturer's recommendations [18] .

Evaluation of biochemistry parameters

The serum was acquired and stored at -80°C until available for further examination after the samples taken from the different groups were taken at the end of the study. Kidney and liver function parameters including urea, BUN, creatinine, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were tested in accordance with the manufacturers' instructions for the reagents (Human diagnostic Company, Germany) [19].

Detection methods for oxidative enzymes

Superoxide dismutase (SOD), an antioxidant enzyme, and malondialdehyde (MDA) lipid peroxidation were assessed in the serum of several animal groups at the end of the experiment [20, 21].

Statistical Analysis

The results' mean and standard deviation (SD) are provided. With a significant level of p ≤0.05, Tukey's test was performed for post-hoc analysis after one-way ANOVA to assess statistical differences. The statistical evaluation was done using the GraphPad prism Version 5 program (San-Francisco, USA).

Results

In-vitro **Antibacterial activity**

L. acidophilus-DSM20079 filtrate and lyophilized cells demonstrated significant antimicrobial properties. The highest antibacterial effects were detected against *Staphylococcus aureus* then *Escherichia coli* and *Micrococcus luteus*, respectively as presented in Table (1).

Table (1): The antibacterial activities of *L. acidophilus*-DSM20079 filtrate and lyophilized cells when tested against selected bacterial strains. Results are expressed as a mean zone of inhibition in mm ± SEM (n = 3) tested by agar well diffusion assay.

Tested microorganisms	Filtrate	Lyophilized cells	Standard Drug
Staphylococcus aureus	16.4 ± 0.9*	12.6 ± 0.8*	28.7 ± 1.2
Bcillus subtilis	11.7 ± 0.6*	8.9 ± 0.7*	29.7 ± 1.5
Micrococcus luteus	14.3± 1.2*	9.5± 0.7*	21.6± 1.3
Pseudomonas aeruginosa	12.6± 0.8*	8.3± 0.5*	17.5± 1.1
Klebsiella pneumoniae	10.9± 0.7*	7.8± 0.6*	22.3± 0.9
Salmonella typhimurium	9.8± 0.9*	8.6± 0.7*	25.4± 1.4
Escherichia coli	15.7± 1.1*	12.2 ± 0.8*	28.1± 1.7

Evaluation of the enzymatic activity of the lipoxygenase enzyme in *L. acidophilus*-DSM20079 lyophilized cells solution and filtrate

L. acidophilus-DSM20079 lyophilized cells solution demonstrated effective suppression of lipoxygenase action when the anti-inflammatory activity of specimens was assessed using lipoxygenase enzymatic inhibition with IC_{50} value of 13.3± 1.4 g/ml. The IC_{50} value for *L. acidophilus*-DSM20079 filtrate was 12.7± 1.6 g/ml, while the ibubrufen standard drug IC_{50} was 1.3 ±0.9 g/ml, as shown in **Figure(1).**

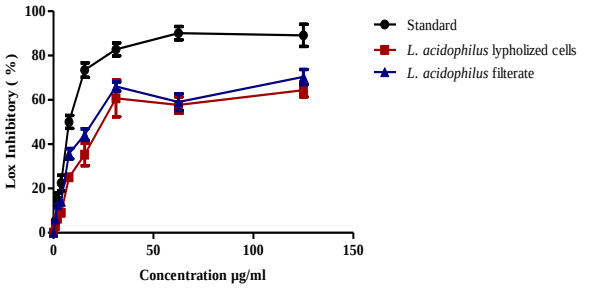
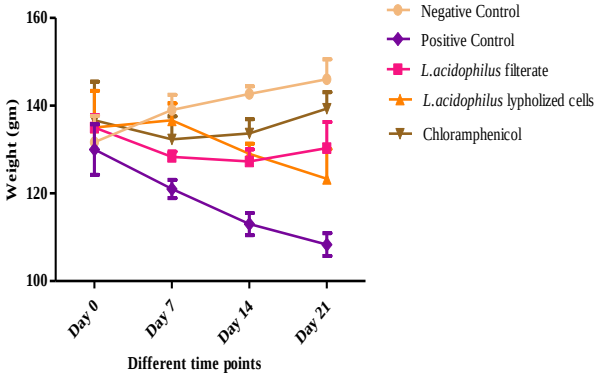
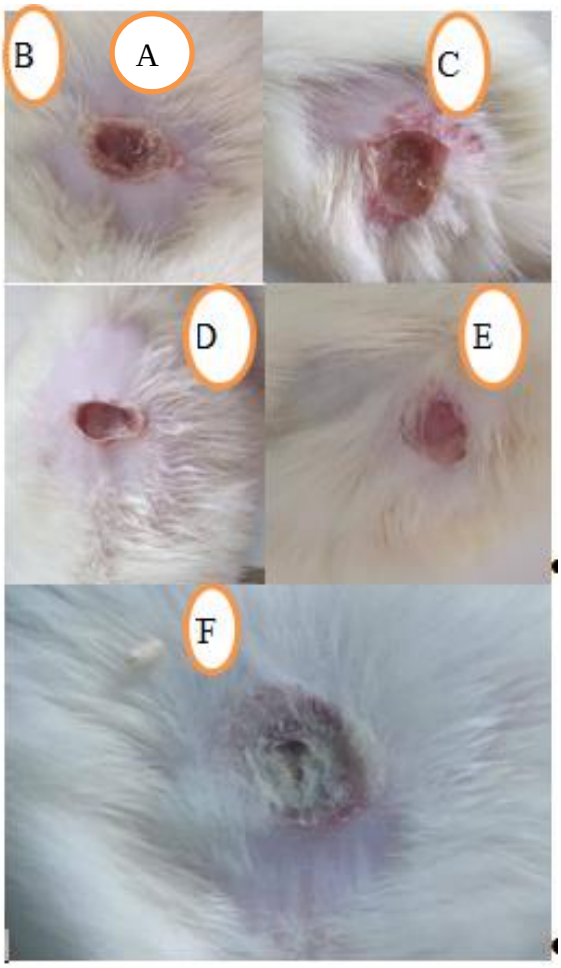


Figure (1): A line graph showing the results of an *in vitro* anti-inflammatory test using Ibuprofen as the reference drug and *L. acidophilus*-DSM20079 solution of lyophilized cells and filtrate. Results are presented as an average SEM (n = 3). For *L. acidophilus*-DSM20079 lyophilized cells, the IC_{50} was 13.3± 1.4 g/ml; for *L. acidophilus*-DSM20079 filtrate, it was 12.7 ±1.6 g/ml; and for standard, it was 1.3± 0.9 g/ml.

Testing body weights and wound healing

The rats were topically medicated with solution of *L. acidophilus*-DSM20079 filtrate, a solution of lyophilized form of *L. acidophilus*-DSM20079, as well as chloramphenicol for 21 days to investigate the outcomes of wound infection. Although the body weights as a clinical symptom of all the animals were the same, the contaminated wound group had a gradual decrease in body weights along testing period and significantly lower body weight than ($p \leq 0.05$) the other-treated and untreated wound groups in the final time point. Additionally, administration of *L. acidophilus*-DSM20079 filtrate had the highest healing activity relative to other groups, where treatment of *L. acidophilus*-DSM20079 filtrate and solution of *L. acidophilus*-DSM20079 lyophilized cells demonstrated a sequential wound healing as depicted in **(Figure2).**





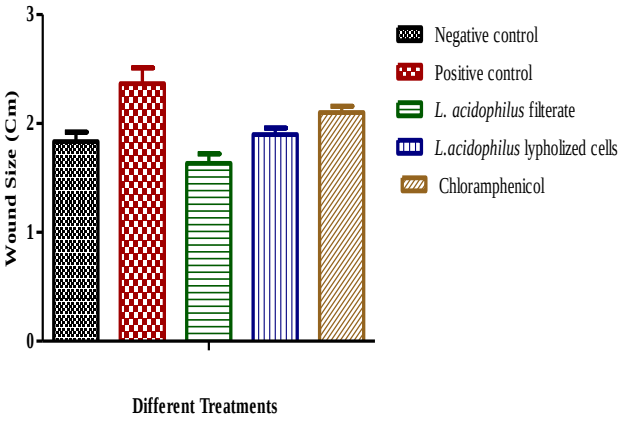
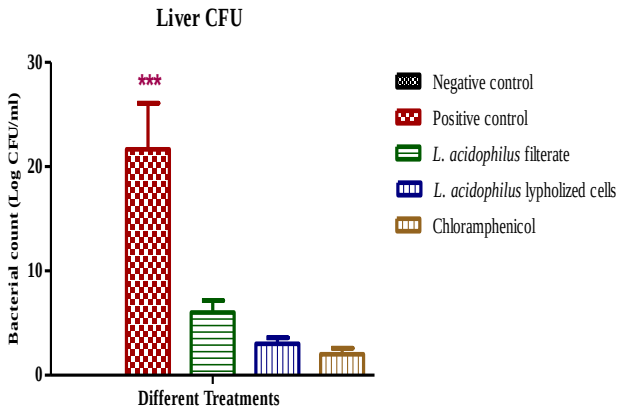
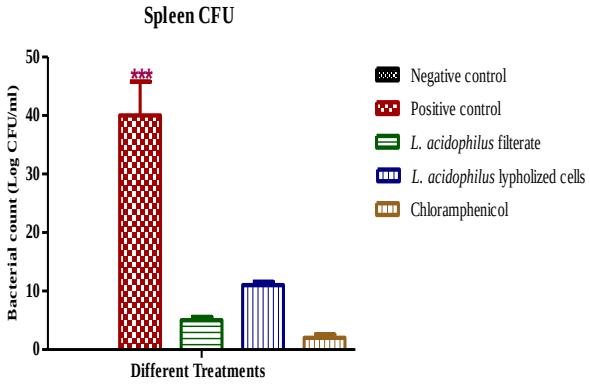


Figure (2) (A) Variations of weights of wounded rats and infected by *S. aureus* and after applying *L. acidophilus*-DSM20079 filtrate, solution of lyophilized *L. acidophilus*-DSM20079 and chloramphenicol, respectively. The size of the wound in **(B)** the un-infected wound and **(C)** the infected wound by *S. aureus*; **(D)** An infected wound that cured by *L. acidophilus*-DSM20079 filtrate; **(E)** the Infected wound attended to by *L. acidophilus*-DSM20079 lyophilized cells solution; **(F)** An infected lesion that was treated with chloramphenicol; and **(G)** data analysis showing variations in wound diameter among the experimental groups (n=3) (Findings are shown as the mean SEM, with a significance level ($p \leq 0.5$).

Examining the bacterial load in various organs and the skin

Swabs of skin from several test groups of animals were used to determine the number of *S. aureus* bacteria, which were subsequently diluted 1:10 in sterile saline and cultivated on mannitol salt agar as the recommended media. When *S. aureus* is used to infect a wound, there is no bacterial growth in the uninfected group, but there is a sharp increase in the number of bacteria. Samples taken from the third group, which employed *L. acidophilus*-DSM20079 filtrate to treat wound infections, revealed a significant decrease in the number of bacteria. where there was no evidence of bacterial development ($p \leq 0.001$). While the fourth group experienced a decrease in bacterial count ($p \leq 0.05$) compared to the second infection group after receiving a suspension of *L. acidophilus*-DSM20079 lyophilized form as demonstrated in **Figure (3).**





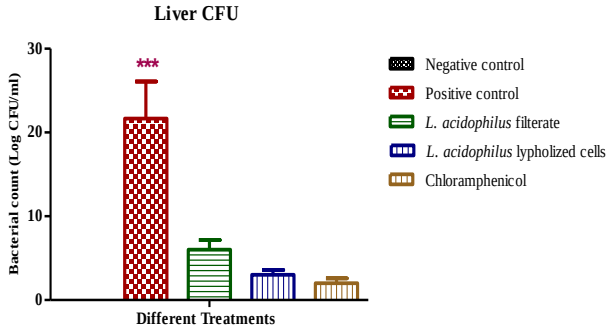


Figure (3): A logarithmic count of *S. aureus* colonies was performed on both skin samples (1:10 dilution) and in several animal species. Where: The first group serves as the negative control, and the second group serves as the positive control, and the wound was infected with 0.5 ml of $X10^3$ *S. aureus* in the second group. The third, fourth, and fifth groups, meanwhile, were treated with 1 ml of *L. acidophilus*-DSM20079 filtrate, 1 ml of *L. acidophilus*-DSM20079 lyophilized cells solution, and chloramphenicol, respectively. Moreover, at the conclusion of the experiment, to find the bacterial count in samples from various groups of liver (1:1 dilution) and spleen (1:1 dilution). (Data were shown as averages with standard deviations; n= 3; $P \leq 0.05$ deemed significant; $P \leq 0.001$ seemed high significant). The use of chloramphenicol causes a progressive decrease in the number of bacteria in skin infections ($p \leq 0.05$) in comparison to the second group (Findings are shown as the mean SEM, with a significance level of (*) p ≤0.5).

Antioxidant enzymes concentrations.

The levels of SOD in the different groups at the end of the experiment were found, as shown in (**Figure (4**).

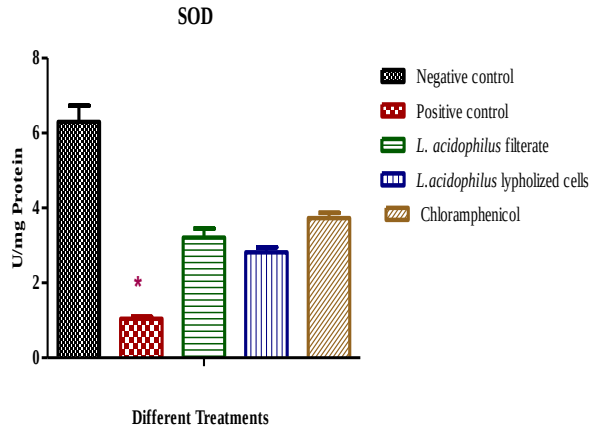


Figure (4): Antioxidant enzymes found in numerous animal groups serum. Rat groups with infected wounds and treated with *L. acidophilus* filtrate-DSM20079, solution of *L. acidophilus* -DSM20079 lyophilized form, and chloramphenicol, respectively, revealed significantly reduced levels of SOD in their serum ($P \leq 0.05$) compared to the negative control group.

The second group in the infected wound group has a much lower SOD level. An increase in SOD levels was seen during treatments with *L. acidophilus*-DSM20079 filtrate, solution of lyophilized cells of *L. acidophilus*-DSM20079, and chloramphenicol ($p \leq 0.05$). No statistically significant difference exists between the different therapeutic groups and the negative control group, suggesting that SOD marginally regains its baseline values between treatments.

Examination of histological sections of different tested groups.

Epidermal hyperplasia in lesions in the dorsal skin of rats was stained with hematoxylin and eosin to examine the histopathological changes. In comparison to the normal wound group, the infected wound group's epidermal thickness was much higher. Additionally, the rats skin treated with chloramphenicol, solution of lyophilized *L. acidophilus*-DSM20079 cells, and filtrate of *L. acidophilus*-DSM20079 showed a reduction of hyperplasia damaged dorsal skin tissues. *L. acidophilus*-DSM20079 filtrate might control the histological alterations in wound infection more effectively than solution of lyophilized probiotic yeast cells at a level comparable to that which chloramphenicol could accomplish, as demonstrated in (**Figure 5**).

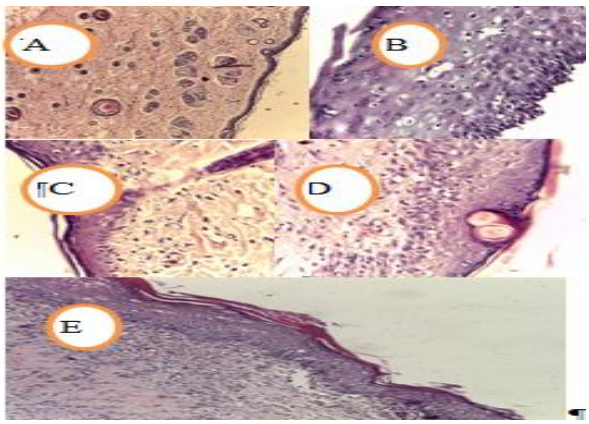


Figure (5) After the research's ending and the sacrifice of the rats, a histological analysis of

the crucial injury issues revealed that **(A)** an uninfected wound contained minor swelling and disintegration of cutaneous layer, and appearance of numerous capillaries (black arrows); **(B)** an infection with *S. aureus* resulted in serious abnormalities and rupture in epidermis black arrow) layer and vacuoles in the dermal layer (green arrow); **(C)** an infection with *S. aureus* and treated by *L. acidophilus*-DSM20079 filtrate showed organized layers of skin; **(D)** an infection with *S. aureus* and treated by a solution of *L. acidophilus*-DSM20079 lyophilized cells where large vacuole could be seen in the generated thin epidermal layer (black arrow) with slight alteration in dermis; **(E)** an infection with *S. aureus* and treated by chloramphenicol where shrink thick formed epidermal layer and regularly structured dermis regularly structured epidermis be seen (Magnification = 40x).

Upon microscopic examination, wherever undeveloped granulation tissue was identified, it was invaded with mast cells, as demonstrated by toluidine blue staining. There were isolated spots of undifferentiated scar tissue that had mast cell infiltration. Using a solution of lyophilized *L. acidophilus*-DSM20079 cells, and filtrate of *L. acidophilus*-DSM20079 revealed a significant increase ($p \leq 0.05$) in mast cells numbers relative to the wound infection untreated group as depicted in (**Figure 6**).

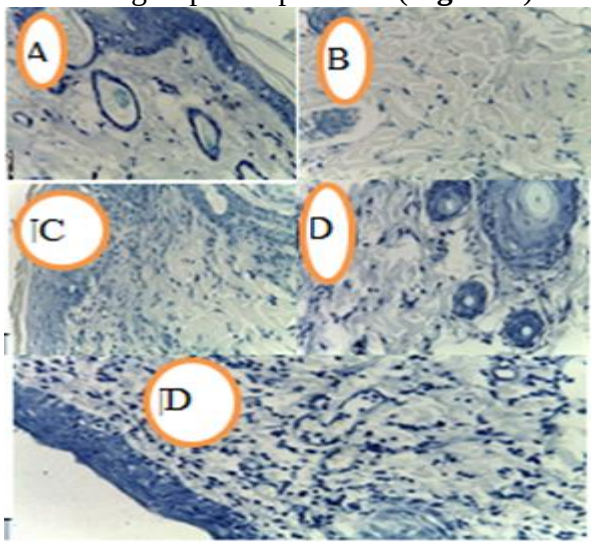


Figure (6): Microstructural examination of toluidine blue-stained histomorphological sections of rat skin from all research groups (x=40). Mast cells are visible in significant numbers in lesions with no infection in (A); they are absent in lesions with bacterial infection in (B); and present in (C), (D), and (E) lesions treated with *L. acidophilus*-DSM20079 filtrate, a solution of *L. acidophilus*-DSM20079 and chloramphenicol, respectively (green arrows). Whereas the mast cells (red arrow) are present in all groups and are mostly found in the endothelial area. They are scarlet purple and have many vesicles. All regions have blue collagen fibers.

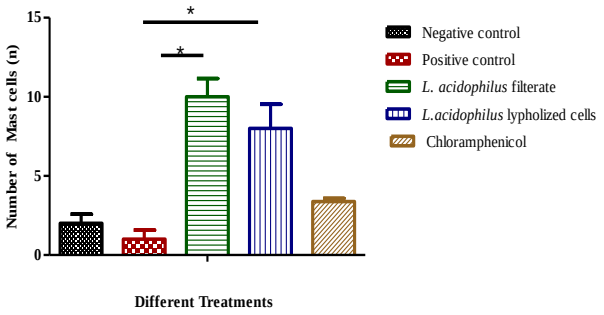


Figure (7): A bar graph illustrating the total ratios of mast cells in the skin of rats across all administrations (n=7 sections/group). The information is displayed as (means± S.D.), where*: significant where $P \leq 0.05$)

Estimation of various interleukins in different groups

The amounts of specific cytokines were measured to examine whether bacterial infection can cause an immunological response in various groups. At the end of experiment serum of sacrificed animals from different groups were analyzed to detect variations in levels of IFN-γ, IL-4, TNF-α, and IL-17.

The use of *S. aureus* to infect the wound was seen. IFN-γ and TNF-α, are among the pro-inflammatory mediators that *S. aureus* significantly upregulated in comparison to the control, uninfected group ($p \leq 0.05$). After application of either *L. acidophilus*-DSM20079 filtrate, suspension of *L. acidophilus*-DSM20079 and chloramphenicol result restore levels of pro-inflammatory mediator that are generally similar to those of the first group and slightly higher than those animals treated with suspension of *L. acidophilus*-DSM20079. The level of IL-17 slightly elevated in the serum second group of animals infected with *S. aureus* while using *L. acidophilus*-DSM20079 filtrate, suspension of *L. acidophilus*-DSM20079 and chloramphenicol lead to reaching level to similar level of the first control group. The readings for *L. acidophilus*-

DSM20079 filtrate are most similar to those for the control and routine standard medication therapy of chloramphenicol. While the second group of animals, when compared to the first control group, showed a substantial drop in the anti-inflammatory mediator IL-4 ($p \leq 0.05$). Additionally, the use of suspension chloramphenicol lead to an increase level of IL-4 in this group on animals higher than control as depicted in (**Figure 8)**

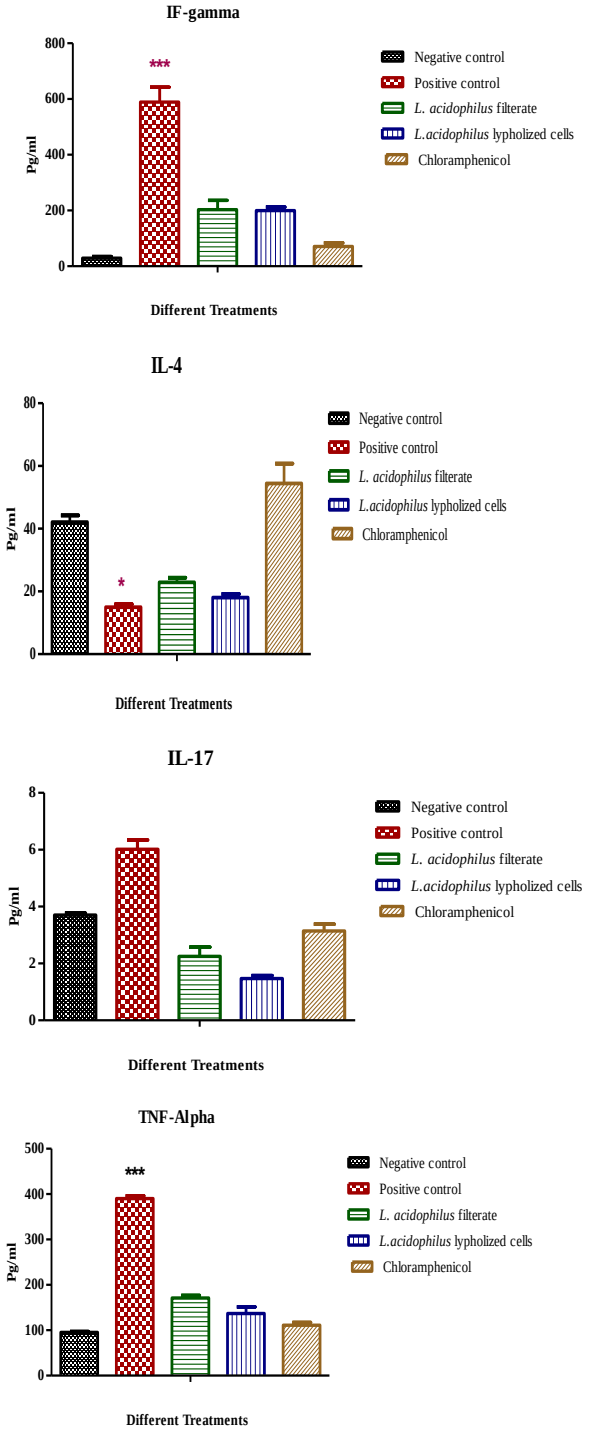
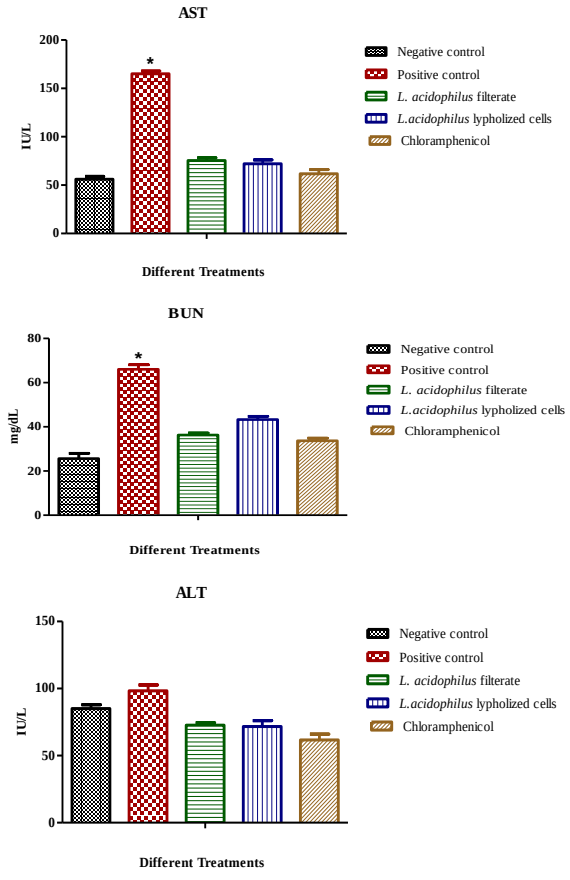


Figure (8) Cytokines measured by ELISA in rats contented regular wound versus infected wound with *S. aureus* and upon treatments of the wound using *L. acidophilus*-DSM20079 filtrate, solution of *L. acidophilus* -DSM20079 lyophilized cells, and chloramphenicol. The level of (A) IFN- γ, (B) IL-4, (C) IL-17, (D) TNF-α, (. in rat serum were detected by ELISA. (Results are presented as means ± SEM, n = 3. *$P < 0.05$, ***$P < 0.001$

Impact of different treatments on various biochemical functions in animals

The results revealed that creatinine, BUN and A.S.T. and levels were significantly elevated in second group contained infected wound relative to uninfected groups ($P < 0.05$). While application of *L. acidophilus*-DSM20079 filtrate, suspension of *L. acidophilus*-DSM20079 and chloramphenicol in infected wound have restore its regular liver and kidney functions as the treatments have anti-inflammatory as well as antibacterial functions which prevent dissemination of bacteria from wound to inside body which lead to load to internal body functions. The results depicted A.L.T functions did not different in all tested groups as illustrated in **(Figure 9)**.



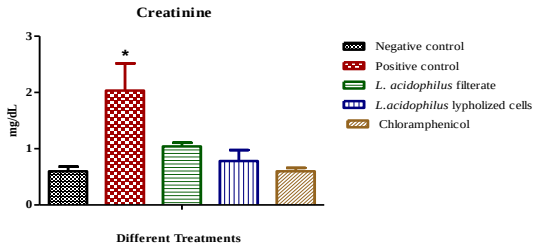


Figure (9) Impact of applying *L. acidophilus*-DSM20079 filtrate, solution of *L. acidophilus* -DSM20079 lyophilized form, and chloramphenicol in rats' skin in serum liver and kidney functions versus regular wound and upon infected wound using *S. aureus*. The level of (A) Creatinine, (B) BUN, (C) ALT, (D) AST in rat serum were determined using biochemical kits. (Results are represented as means ± S.E.M. **P* < 0.05).

Discussion

It has been documented that some resistant clinical bacterial isolates are susceptible to the inhibitory effects of lactic acid bacteria. Numerous species of the genus *Lactobacillus* can colonize in particular body regions, such as the mouth, where they are crucial to the direct inhibition of pathogens [22, 23]. It turns out that the therapeutic efficacy of *Lactobacillus* strains versus infectious agents is multifunctional and involves the formation of compounds like bacteriocin, hydrogen peroxide, lactic acid, and unidentified heat-stable chemicals that are not lactic acid as well as enhancing immune system [24]. In this report antibacterial activity of *Lactobacillus acidophilus*-DSM20079 has been tested. [25, 26] reported benefits of *Lactobacilli* include their ability to prevent the growth of gram-negative and gram-positive harmful bacteria. Moreover, who confirmed antimicrobial action towards *E. coli*.

A topic of current attention is the use of unconventional treatments for inflammatory illnesses, such as the ingestion of fermenting dairy or non-milk based foods [27]. In the present report *Lactobacillus acidophilus*-DSM20079 filtrate showed promising *in vitro* anti-inflammatory action and higher than solution of lyophilized *Lactobacillus acidophilus*-DSM20079. In accordance with [28] who reported that various L. acidophilus concentrations trigger inflammatory response with different values and regulate the development and eradication of biofilms.

In the present investigation application of either *L. acidophilus*-DSM20079 filtrate, suspension of *L. acidophilus*-DSM20079 enhanced wound healing same like treating using chloramphenicol which may positively impact the appetite of animals and body weights relative to untreated animals. However, [29] who reported that fermentation of Mulberry by *Lactobacillus acidophilus* A4 lessened inflammation in the induced rat model and did not impact weights.

Bacterial loads have been significantly decreased upon using either *L. acidophilus*-DSM20079 filtrate or suspension of *L. acidophilus*-DSM20079 relative to using standard drug in the wound infection model revealing their pleotropic action. *L. acidophilus* does not seriously endanger the hosts and, in addition, has been observed to be crucial in preventing an overabundance of potential hazardous bacteria in the intestines [30, 31]. Remedies to the topically applied medicines are of considerable interest since resistance to antibiotics is a serious danger that people are becoming increasingly concerned about. Through inducing the formation of immune cells and/or competitive exclusion of microorganisms that cause skin infections, probiotics are known to improve host health and skin healing [32] [33] .

Understanding the microstructure and physiology of skin is crucial for predicting and enhancing wound closure [34]. In the present work histological examination of skin from different treatments revealed that the application of *L. acidophilus*-DSM20079 filtrate, suspension of *L. acidophilus*-DSM20079 enhance the wound healing process through re-epithelialization process and down regulate inflammation process. In the present study assessment of variations has been done at the end of experiment in rat infection model. Clinical wounds in persons and experimental wounds in lab rats come in two different forms: Individuals heal mostly by re-epithelialization while rats repair by compression. Second, it is feasible to observe changes that occur in size and form in all of the rats at once in an experimental wound [35, 36]. *Lactobacillus* reported to increase healing rate through optimization of hemostasis, decrease an inflammation inside the tissue, fibroblast

proliferation, collagen deposition, remodeling by collagen cross-linking and scar maturation. Inflamed regions exhibits hypoxia frequently, which enhances the generation of reactive oxygen compounds and free radical's molecules nearby, leading to incorrect or protracted tissue repair [37]. In this work using of *L. acidophilus*-DSM20079 filtrate, suspension of *L. acidophilus*-DSM20079 upregulate superoxide dismutase enzyme and decrease secretion of malondialdehyde. The present results same line with [38] reported that different forms of probiotics could impact the healing mechanism through NO synthestase.

Mast cells were shown to be important for the activation of neutrophils and macrophages and to act as regulators of rapid inflammatory process to healing process [39]. Keratinocytes are stimulated by mediators produced by Mast Cells, such as epidermal growth factor [40]. There is still a lot that do not comprehend about the action mechanism and the way these cells behave *in vivo*, although the present knowledge of the role that Mast Cells conduct and their participation throughout many facets of healing [41]. In this study application of *L. acidophilus*-DSM20079 filtrate, suspension of *L. acidophilus*-DSM20079 and chloramphenicol led to elevation of number of mast cells which enhance healing process in wound infection model. The production of cytokines and chemokines by retained platelets in the wound area, including tumor necrosis factor-alpha, IL-6, and many others, draw neutrophils to the injured area. Neutrophils phagocytose and break down pathogens, and tissue fragments in the wound. They also trigger an immune response that draws in other neutrophils and inflammatory cytokines [42]. In this study administration of *L. acidophilus*-DSM20079 filtrate, suspension of *L. acidophilus*-DSM20079 and chloramphenicol affect Th1, Th2 and Th17 cytokines where derive the protective cytokines including IFN-γ, TNF-α, and IL-17 to optimal levels and increase secretion of anti-inflammatory cytokine (IL-4) which enhance healing process. In a skin experimental model, *Staphylococcus aureus* lesions that are applied to the skin layer can spread to various organs. Blood cultures from these animals came back negative, and surface administration improved the effectiveness of the spread. *S. aureus* is susceptible of hematogenous spread, but it can also travel non-bacteremically from the epidermis to deep regions. The germs in this instance seem to move straight from the top layer of the skin to the deeper tissues underneath [43]. In the current study administration of *L. acidophilus*-DSM20079 filtrate, suspension of *L. acidophilus*-DSM20079 and chloramphenicol have protective effect towards wound healing as well as antimicrobial effect towards microbial infection by *S. aureus* and prevent dissemination of bacteria to internal organs and regulate liver and kidney functions to lower levels relative than untreated infected wound group. In the present work using *L. acidophilus*-DSM20079 filtrate, suspension of *L. acidophilus*-DSM20079 can be successive in healing process in wound infection model with suggested multifunction in anti-inflammatory, antimicrobial and regulation of cellular mediators to be applied with high safety profile.

4. References

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