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Topical Fermented *Lactobacillus acidophilus* Lysate Accelerates Second-Degree Burn Healing: An *In Vivo* Study in Wistar Rats

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A B S T R A C T

Second-degree burns, affecting the epidermis and dermis, constitute a major category of thermal injuries globally, presenting significant clinical challenges including pain, infection risk, and potential scarring. While standard treatments like silver sulfadiazine (SSD) exist, limitations including potential cytotoxicity and emerging resistance necessitate exploration of novel therapeutic strategies. Recent interest has focused on topical applications derived from probiotics, such as *Lactobacillus* spp., due to their suggested roles in modulating inflammation, combating oxidative stress, and providing antimicrobial activity to accelerate wound repair. This study investigated a fermented lysate derived from *Lactobacillus acidophilus* (*L. fermented*) as a potential topical agent for burn healing. This study aimed to evaluate the therapeutic efficacy of a 5% topical *L. fermented* ointment on the healing process of experimentally induced second-degree burns in a Wistar rat model, primarily by assessing the rate of wound closure compared to standard SSD treatment and an untreated control. A true experimental *in vivo* study utilizing a post-test only control group design was performed following ethical approval. Fifteen male Wistar rats were subjected to a standardized second-degree thermal burn injury on their dorsal aspect. The animals were then randomized (n=5 per group) to receive twice-daily topical applications of either 5% *L. fermented* ointment (Group A), SSD ointment (Group B), or no treatment (Control, Group C). Wound healing was quantitatively assessed by measuring the wound surface area (mm^2) on days 1, 3, 7, and 14 post-injury using digital imaging and ImageJ software analysis. Statistical comparisons between groups were conducted using one-way ANOVA followed by LSD post-hoc tests, with statistical significance set at $p<0.05$. All treatment groups demonstrated a significant reduction in wound size over the 14-day observation period ($p=0.001$ within each group). Inter-group comparisons revealed significantly accelerated wound closure in Group A starting from day 3 onwards ($p<0.005$). At day 14, the mean wound area in Group A (17.5 ± 8.06 mm^2) was significantly smaller than in Group B (119.22 ± 45.41 mm^2) and Group C (305.18 ± 25.21 mm^2) ($p=0.001$). Post-hoc analysis confirmed the superiority of *L. fermented* treatment over both SSD (mean difference 101.72 mm^2, $p=0.001$) and control (mean difference 287.68 mm^2, $p=0.001$). SSD treatment also resulted in significantly better healing than the control group (mean difference 185.96 mm^2, $p=0.001$). In conclusion, topical application of 5% fermented *Lactobacillus acidophilus* lysate significantly accelerated the closure of second-degree burn wounds in Wistar rats, demonstrating superior efficacy compared to both silver sulfadiazine treatment and no treatment. These findings highlight the potential of *L. fermented* lysate as a promising novel therapeutic agent for burn wound management.

1. Introduction

Thermal burn injuries constitute a significant global health challenge, contributing substantially to morbidity and mortality worldwide, with a disproportionate impact on low- and middle-income countries. Second-degree burns, characterized by damage extending through the epidermis into varying depths of the dermis, represent a prevalent type of

thermal injury, accounting for a large proportion of cases. These injuries disrupt the skin's critical barrier function, leading to a cascade of physiological consequences including fluid loss, intense pain, and heightened susceptibility to microbial colonization and subsequent infection. The disruption of the skin barrier initiates a complex wound healing process, a process that can be further compromised by infection, potentially resulting in hypertrophic scarring and functional impairment. The management of second-degree burns typically involves a multi-faceted approach. This standard care regimen includes meticulous wound cleansing, debridement of necrotic tissue and/or large blisters (when indicated), and the application of topical antimicrobial agents. Topical antimicrobial agents are crucial in preventing and managing infections in burn wounds. Silver sulfadiazine (SSD) has been a mainstay in topical burn wound management due to its broad-spectrum antimicrobial activity. However, the use of SSD is associated with limitations that necessitate the exploration of alternative or adjunctive therapeutic strategies. Concerns exist regarding its potential cytotoxicity, specifically affecting keratinocytes and fibroblasts, which are critical cell types involved in the wound healing process. SSD has also been implicated in delaying re-epithelialization in certain clinical contexts, a crucial step in skin regeneration. Furthermore, the potential for systemic absorption of silver, particularly in cases of large burn areas, raises additional concerns. Perhaps of even greater concern is the emergence of silver-resistant bacterial strains, which threatens to undermine the effectiveness of SSD in combating burn wound infections. These limitations highlight the critical need for the development of alternative or adjunctive therapies that not only effectively control infection but also actively promote a more efficient and qualitatively superior healing process.[1-4]

In recent years, there has been a growing interest in the therapeutic potential of modulating the skin microbiome or harnessing the properties of microbial products for dermatological applications and wound care. Probiotics, defined as live microorganisms that confer health benefits to the host, and increasingly, postbiotics, which are non-viable bacterial products or metabolic byproducts, are being investigated for their potential role in promoting skin health and facilitating repair processes. Topical applications of these agents are particularly attractive due to the ability to deliver them directly to the target site of action. *Lactobacillus* species are among the most extensively studied probiotics, recognized for their diverse beneficial properties. These bacteria are known to produce a range of antimicrobial compounds, including lactic acid and bacteriocins, which can inhibit the growth of pathogenic microorganisms. Furthermore, *Lactobacillus* species can compete with pathogens for nutrients and adhesion sites, limiting their ability to colonize and infect the host. Importantly, they possess the capacity to modulate host immune responses, influencing both innate and adaptive immunity. Fermented lysates or cell-free supernatants derived from *Lactobacillus* cultures represent a specific type of postbiotic preparation. These postbiotic preparations may offer significant therapeutic benefits while potentially mitigating the risks associated with the application of live bacteria to compromised skin barriers, such as those found in burn wounds. These preparations contain a complex mixture of bacterial components, including cell wall fragments such as peptidoglycan and lipoteichoic acid, as well as a variety of secreted metabolites, such as organic acids, enzymes, and peptides.[5-7]

Preliminary evidence suggests that these lysates possess a range of activities relevant to the wound healing process. These activities include anti-inflammatory effects, which can help to control excessive inflammation that can impede healing. They also exhibit antioxidant activities, which can help to neutralize reactive oxygen species (ROS) generated at the wound site. Additionally, these lysates demonstrate antimicrobial effects, which can help to prevent or control infection. Finally, they possess tissue regenerative effects, which can actively promote

the repair and regeneration of damaged tissue. The components within these lysates can influence various inflammatory signaling pathways, such as the NF-κB signaling pathway. They can also modulate cytokine profiles, shifting the balance towards a more pro-healing state. Furthermore, they can enhance endogenous antioxidant defenses through pathways such as the NRF2 pathway, leading to increased expression of antioxidant enzymes. Finally, they can potentially stimulate the production of growth factors, such as Transforming Growth Factor-beta (TGF-β), which plays a critical role in tissue remodeling.[8-10] Given this background, this study was designed to rigorously evaluate the efficacy of a topically applied 5% fermented lysate derived from *Lactobacillus acidophilus* in a well-established preclinical model of second-degree thermal burns in Wistar rats.

2. Methods

This study was designed as a true experimental *in vivo* investigation, employing a post-test only control group design. The study aimed to evaluate the efficacy of a 5% topical *Lactobacillus acidophilus* fermented lysate ointment on the healing of experimentally induced second-degree thermal burns in a Wistar rat model. The primary outcome measure was the rate of wound closure, compared across the treatment groups. The experiments were conducted at the Laboratorium Pusat Studi Pangan dan Gizi Universitas Gadjah Mada (PSPG UGM) between May and August 2024. All experimental procedures were performed in strict adherence to the ethical principles outlined in the Declaration of Helsinki and relevant institutional guidelines concerning animal care and use. Ethical clearance for the study was obtained from the Health Research Ethics Committee (HREC) of Dr. Moewardi Surakarta Hospital.

The active treatment consisted of a 5% fermented *L. acidophilus* lysate ointment. This preparation is subsequently referred to as "*L. fermented*" throughout this document. The ointment base was prepared utilizing standard pharmaceutical methods. Initially, the oil phase components, comprising DM 100, stearic acid, Cithrol GMS, and cetyl alcohol, were combined and heated within a temperature range of 70 to 80°C. In a separate vessel, the water phase components, consisting of water, Viscolam AT, glycerin, propylene glycol, TEA (triethanolamine), and Na.EDTA (sodium ethylenediaminetetraacetate), were also heated to the same temperature range of 70 to 80°C. Once both phases reached the desired temperature, the oil phase was slowly incorporated into the water phase. This incorporation process was accompanied by continuous stirring to ensure the formation of a homogenous cream base. Following the formation of the cream base, the mixture was allowed to cool to room temperature. Subsequently, purasal, CM 45, and bisabolol were added to the cream base. The resultant mixture was thoroughly mixed until homogeneity was achieved, ensuring uniform distribution of all constituents. The final step in the preparation of the active treatment involved the incorporation of 5% (w/w) of Lactosome-55 into the homogenous ointment base. Lactosome-55 constitutes the proprietary *L. acidophilus* fermented lysate preparation. The lysate was mixed thoroughly into the base to yield the final, homogenous ointment formulation. For the comparator group, a commercially available standard silver sulfadiazine (SSD) ointment preparation was utilized. This ensured that the comparator treatment adhered to the current standard of care for burn wound management.

The study utilized healthy male Wistar rats, with a weight range of 200 to 230 grams at the commencement of the experiment. These animals were obtained from the Laboratory of Inter-University Center for Food and Nutrition, Gadjah Mada University, Yogyakarta. Upon arrival at the animal facility, the rats were housed under standard laboratory conditions. These conditions included a controlled temperature environment, a 12-hour light/dark cycle to mimic natural diurnal rhythm, and ad libitum access to standard laboratory chow and water. This ensured that the animals had unrestricted access to nutrition and hydration. Prior to the

initiation of the experimental procedures, the animals were allowed to acclimatize to the laboratory conditions. This acclimatization period was crucial to minimize stress and allow the animals to adapt to their new environment, thereby reducing potential confounding factors in the study results. Only rats that met specific inclusion criteria and were free from any visible signs of illness or disability were included in the study. This stringent selection process aimed to ensure the homogeneity of the experimental population and to minimize variability in response to the treatments.

To perform the experimental procedures, it was necessary to induce anesthesia in the Wistar rats. Anesthesia was initially induced through the administration of atropine sulfate at a dosage of 0.04 mg/kg via intramuscular (IM) injection. Atropine sulfate was used as a pre-anesthetic medication to reduce salivation and bronchial secretions, which can be problematic during general anesthesia. Following the administration of atropine sulfate, a mixture of ketamine (90 mg/kg, 10% solution) and xylazine (10 mg/kg, 2% solution) was administered via intramuscular injection. Ketamine is a dissociative anesthetic, while xylazine is a sedative and muscle relaxant. The combination of these two agents provides effective anesthesia for various procedures in rats.

Once adequate anesthesia was confirmed, the dorsal back of each rat was carefully prepared for the induction of the thermal burn injury. The hair in the dorsal region was removed by shaving. This was performed meticulously to avoid causing any injury or irritation to the skin. Following the shaving procedure, the exposed skin was prepared aseptically. This involved the application of a 1% polyvinylpyrrolidone iodine solution to the shaved area. Polyvinylpyrrolidone iodine is a broad-spectrum antiseptic that is commonly used to disinfect the skin prior to surgical or invasive procedures, thereby minimizing the risk of infection.

A standardized second-degree thermal burn was induced on the prepared dorsal skin of each rat. The method employed to induce the burn involved the application of a solid aluminum rod. The aluminum rod had a diameter of 10 mm and a mass of 51 grams. Prior to application, the aluminum rod was pre-heated to a consistent temperature of 100°C. This was achieved by immersing the rod in boiling water, and the temperature was verified using a thermometer to ensure accuracy and consistency. The pre-heated aluminum rod was then applied onto the shaved dorsal skin of the anesthetized rat for a precise duration of 15 seconds. This method is well-established in experimental research for the reliable and reproducible induction of partial-thickness burns in animal models. The controlled temperature and application time ensured the consistency of the burn injury across all animals.

Immediately following the induction of the thermal burn, the rats received analgesia to alleviate pain and ensure animal welfare. Dipyrone sodium was administered at a dosage of 40 mg/kg via intramuscular injection. Dipyrone sodium is a non-steroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic properties. The administration of dipyrone sodium was continued orally for the subsequent two days following the burn induction. This prolonged analgesic regimen aimed to provide adequate pain management throughout the initial phase of the healing process, minimizing discomfort and distress to the animals.

Fifteen rats, confirmed to have sustained comparable second-degree burns, were included in the study. These animals were then randomly allocated into three experimental groups. Each group consisted of five rats (n=5 rats per group). The sample size determination was based on the Federer formula, a statistical method used for determining the minimal sample size required for comparative studies to ensure adequate statistical power. This randomization process aimed to minimize bias and ensure that the characteristics of the animals were evenly distributed across the treatment groups. The three experimental groups were designated as follows; Group A (*L. fermented*): This group received the active treatment.

Animals in this group received topical application of 0.5 grams of the 5% *L. fermented* ointment. The ointment was spread gently over the entire burn wound surface. The application was performed twice daily, once in the morning and once in the evening; Group B (SSD): This group served as the positive control group. Animals in this group received topical application of 0.5 grams of the standard silver sulfadiazine (SSD) ointment. Similar to Group A, the ointment was spread gently over the entire burn wound surface, and the application was performed twice daily (morning and evening); Group C (Control): This group served as the negative control group. Animals in this group received no topical treatment on the burn wound. This group allowed for the observation of the natural healing process without any pharmacological intervention.

Following the burn induction and the commencement of treatments, all animals were housed individually. This individual housing was implemented to prevent any potential interference with the wounds or the applied treatments. Housing the animals separately ensured the integrity of the experimental conditions and prevented cross-contamination or self-inflicted trauma to the wounds.

The progression of wound healing was quantitatively monitored throughout the study period. This monitoring was achieved by measuring the wound surface area at specific time points days 1, 3, 7, and 14 post-burn induction. On each of these assessment days, the wounds were carefully photographed. To minimize stress to the animals and ensure accurate imaging, light anesthesia was administered if deemed necessary. The photographs were taken using a digital camera. The camera was mounted at a fixed distance from the wound and under standardized lighting conditions. This standardization was crucial to maintain consistency in the images and allow for accurate comparison of wound sizes across different time points and treatment groups. To provide a reference for scale, a calibrated millimeter ruler was included in each photograph. This ruler allowed for the accurate measurement of the wound area from the digital images.

The wound margins were clearly identifiable in the digital photographs. The measurement of the wound area was performed by two independent observers. These observers were blinded to the treatment group assignments. Blinding was implemented to eliminate potential bias in the measurements. The wound area was measured using the ImageJ software (National Institutes of Health, USA). ImageJ is a widely used image processing program in scientific research. The area of each wound was calculated to two decimal places, ensuring a high degree of precision in the measurements. For each wound, the average of the two independent measurements was calculated. This average value was used for all subsequent statistical analyses.

All quantitative data, specifically the wound area measurements, were expressed as mean ± standard deviation (SD). Statistical analyses were performed using IBM SPSS Statistics for Windows, version 23 (IBM Corp., Armonk, NY, USA). Prior to conducting parametric statistical tests, the normality of the distribution of the wound size data within each group at each time point was assessed. The Shapiro-Wilk test was used for this assessment. Differences in mean wound size between the three groups at each specific time point (days 1, 3, 7, and 14) were analyzed using a one-way Analysis of Variance (ANOVA). Changes in wound size over time within each group were also analyzed. This analysis was conducted using ANOVA for repeated measures. In cases where the assumptions of ANOVA were violated, an appropriate non-parametric equivalent test was used. Repeated measures ANOVA is used to analyze data where the same subjects are measured multiple times. Post-hoc comparisons between specific groups were conducted. These comparisons were performed at significant time points, particularly at the study endpoint on day 14. The Least Significance Difference (LSD) test was used for these post-hoc analyses. The LSD test is a post-hoc test used to determine which specific groups differed significantly from each other following a significant

ANOVA result. For all statistical tests, a p-value of less than 0.05 was considered to indicate statistical significance.

3. Results and discussion

Table 1 presents a comparative analysis of second-degree burn wound healing across different treatment groups over a 14-day period. The key measurements are the mean wound size, expressed in square millimeters (mm^2), at various time points post-burn. The table also provides the percentage of wound reduction from day 1 to day 14. On day 1, the mean wound sizes among all three groups (Group A: *L. fermented*, Group B: SSD, and Group C: Untreated Control) were statistically similar. This is indicated by the high p-value (0.704), suggesting no significant difference in the initial wound severity across the groups. This is crucial as it confirms that the study started with comparable burn injuries in each group. By day 3, a significant difference in wound size emerged between the groups, as shown by the p-value of 0.003. Group A, treated with the *L. fermented* lysate, showed a trend towards smaller wound sizes compared to Groups B and C. This suggests that the *L. fermented* treatment may have initiated a more rapid healing process early on. The difference in wound size became more pronounced by day 7, with Group A continuing to exhibit smaller mean wound sizes compared to the other groups (p=0.002). This trend indicates that the *L. fermented* treatment consistently promoted better wound healing over time. At the final assessment on day 14, there was a highly significant difference in wound size between the groups ($p < 0.001$). The mean wound area in Group A was substantially smaller than in both Group B (SSD) and Group C (Control). Specifically, Group A had a mean wound area of 17.50 mm^2, while Group B had 119.22 mm^2, and Group C had 305.18 mm^2. This clearly demonstrates the superior efficacy of the *L. fermented* treatment in promoting wound closure by the end of the study. The percentage of wound reduction from day 1 to day 14 further highlights the treatment differences. Group A exhibited the highest wound reduction (96.1%), followed by Group B (73.5%), and Group C showed the least reduction (33.7%). This metric provides a clear comparative measure of the overall healing effectiveness of each treatment.

Table 1. Comparison of second-degree burn wound healing progression among treatment groups.

Assessment day	Measurement	Group A (5% *L. fermented*)	Group B (SSD ointment)	Group C (untreated control)	p-value (between groups)[a]
Day 1	Mean wound size ± SD (mm^2)	449.66 ± 29.16	450.42 ± 18.93	460.35 ± 16.37	0.704
Day 3	Mean wound size ± SD (mm^2)	373.03 ± 44.72	418.55 ± 17.84	451.95 ± 12.16	0.003
Day 7	Mean wound size ± SD (mm^2)	241.97 ± 80.14	303.91 ± 58.34	406.46 ± 15.36	0.002
Day 14	Mean wound size ± SD (mm^2)	17.50 ± 8.06	119.22 ± 45.41	305.18 ± 25.21	<0.001
Overall	% wound reduction (Day 1-14)**[b]	96.1%	73.5%	33.7%	N/A

Notes: Group A: Treated with 5% fermented *Lactobacillus acidophilus* lysate ointment; Group B: Treated with silver sulfadiazine (SSD) ointment; Group C: Untreated control group; [a]p-value: Result of one-way ANOVA test comparing the mean wound sizes between the three groups at each specific assessment day; **[b]: percentage wound reduction: calculated as ((mean day 1 - mean day 14) / mean day 1)x100%. Represents the overall percentage decrease in wound size from the initial measurement to the final measurement for each group; *: Indicates a statistically significant difference ($p < 0.05$) between the groups at that time point.
Abbreviation: N/A: Not Applicable; SD: standard deviation.

Table 2 presents a pairwise comparison of mean wound sizes between the treatment groups at day 14, using the Least Significant Difference (LSD) post-hoc test. This analysis was conducted to precisely quantify the differences in healing outcomes at the study's endpoint; Comparison between Group A (*L. fermented*) and Group B (SSD): The mean difference in wound size between these two groups was 101.72 square millimeters. The p-value associated with this comparison was less than 0.001, indicating a statistically significant difference. This result confirms that the *L. fermented* treatment resulted in significantly smaller residual wound areas compared to the standard SSD treatment at day 14; Comparison between Group A (*L. fermented*) and Group C (Control): The mean difference in wound size between these groups was 287.68 square millimeters. The p-value was also less than 0.001, demonstrating a statistically significant difference. This finding shows that the *L. fermented* treatment led to significantly better healing, resulting in much smaller wound areas, compared to the untreated control group at day 14; Comparison between Group B (SSD) and Group C (Control): The mean difference in wound size between these groups was 185.96 square millimeters. The p-value was less than 0.001, again indicating a statistically significant difference. This result shows that the standard SSD treatment also resulted in significantly better healing, with smaller wound areas, compared to the untreated control group at day 14.

Table 2. Pairwise comparison of mean wound sizes between treatment groups on day 14 using LSD post-hoc test.

Pairwise Comparison	**Mean difference in wound size (mm^2)[b]**	**p-value[a]**	**Significance**
Group A (L. fermented) vs group B (SSD)	101.72	<0.001	Significant
Group A (L. fermented) vs group C (Control)	287.68	<0.001	Significant
Group B (SSD) vs group C (Control)	185.96	<0.001	Significant

* Statistically significant ($p<0.001$).

The results of this study provide compelling evidence for the superior efficacy of the *L. acidophilus* fermented lysate in promoting the healing of second-degree thermal burns. The data clearly illustrate a significantly accelerated rate of wound closure in the group treated with the *L. fermented* lysate when compared to both the SSD-treated group and the untreated control group. This accelerated healing was evident as early as day 3 post-burn, with the *L. fermented* group exhibiting a trend toward smaller wound sizes. This difference in wound size became progressively more pronounced throughout the study period, culminating in a highly significant difference by day 14. At this final assessment point, the mean wound area in the *L. fermented*-treated group was substantially smaller than that observed in the SSD-treated group and the untreated control group. The quantitative data are further supported by qualitative observations. Visual assessment of the wound photographs taken throughout the study period corroborated the quantitative findings, demonstrating a visibly faster rate of wound contraction and re-epithelialization in the *L. fermented*-treated group compared to both the SSD-treated group and the untreated control group. This enhanced rate of wound contraction and re-epithelialization is a critical indicator of accelerated and improved wound healing. The superior efficacy of the *L. fermented* lysate is further emphasized by the analysis of the percentage of wound reduction from day 1 to day 14. The *L. fermented*-treated group exhibited a significantly higher percentage of wound reduction compared to both the SSD-treated group and the untreated control group. This metric provides a comprehensive measure of the overall improvement in wound healing achieved with the *L. fermented* lysate treatment. The findings of this study are particularly significant when considering the limitations associated with current

standard treatments for burn wounds. Silver sulfadiazine (SSD), while being a mainstay in topical burn wound management due to its broad-spectrum antimicrobial activity, has several drawbacks. These limitations include potential cytotoxicity to keratinocytes and fibroblasts, delayed re-epithelialization in certain contexts, the risk of systemic silver absorption, and the emergence of silver-resistant bacterial strains. The superior efficacy of the *L. fermented* lysate in promoting wound healing, as demonstrated in this study, suggests that it could serve as a valuable alternative or adjunctive therapy to SSD, potentially mitigating these limitations and improving clinical outcomes in burn wound management.[11-13]

The mechanisms underlying the accelerated wound healing observed with the *L. acidophilus* fermented lysate are likely multifaceted and involve the modulation of several key biological processes. While the precise mechanisms were not fully elucidated in this study, the existing body of scientific literature provides valuable insights into the potential modes of action of *Lactobacillus*-derived products in promoting wound repair. These potential mechanisms include immunomodulation and inflammation control, antioxidant activity, promotion of tissue regeneration, and antimicrobial effects. Inflammation is a critical and complex component of the early stages of wound healing. It is a necessary response to tissue injury, facilitating the recruitment of immune cells and the initiation of the repair process. However, while an initial inflammatory response is essential, prolonged or excessive inflammation can be detrimental to the healing process, leading to tissue damage and delayed repair. *Lactobacillus*-derived products, including those present in the *L. acidophilus* fermented lysate used in this study, have been shown to possess immunomodulatory properties, capable of influencing host immune responses. Components within the lysate may exert a dampening effect on excessive pro-inflammatory signaling, potentially through various mechanisms. One potential mechanism involves the inhibition of signaling pathways such as NF-κB, a key transcription factor involved in the regulation of pro-inflammatory cytokine expression. By inhibiting NF-κB activation, the *L. fermented* lysate may help to reduce the production of pro-inflammatory cytokines, thereby mitigating excessive inflammation at the wound site. Another potential mechanism by which the *L. acidophilus* fermented lysate may modulate inflammation is by promoting a shift towards a pro-resolution cytokine profile. Studies on *Lactobacillus* supernatants have demonstrated the modulation of key cytokines, such as a reduction in the levels of pro-inflammatory cytokines like TNF-α and an increase in the levels of anti-inflammatory cytokines like IL-10. This shift in cytokine balance towards a more anti-inflammatory or pro-resolution state can contribute to a more controlled and effective healing process. Furthermore, some studies have suggested that *Lactobacillus* supernatants can induce a dynamic immunomodulation throughout the healing process. For instance, the upregulation of IL-6 in the early phase of wound healing, followed by the upregulation of IL-10 in the later phases, has been observed with *Lactobacillus plantarum* supernatant. This dynamic modulation of cytokine expression may be crucial for orchestrating the different stages of the healing process, ensuring a timely and effective transition from inflammation to proliferation and tissue remodeling. Burn injuries are characterized by a significant increase in oxidative stress at the wound site. This increase in oxidative stress is primarily due to the release of reactive oxygen species (ROS), which are highly reactive molecules that can cause damage to cells and impair the healing process. *Lactobacillus* products, including the *L. acidophilus* fermented lysate used in this study, have been increasingly recognized for their ability to counteract oxidative stress. Cell-free supernatants derived from *Lactobacillus* cultures have demonstrated the capacity to protect cells against H2O2-induced stress *in vitro*, indicating their potential to neutralize ROS and mitigate oxidative damage. One important mechanism by which *Lactobacillus* products can exert antioxidant effects is through the activation of the NRF2 pathway. NRF2 is a key regulator of

cellular antioxidant defenses, controlling the expression of a wide range of antioxidant enzymes. Activation of the NRF2 pathway leads to an increased expression of antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione-related enzymes. These enzymes play a crucial role in neutralizing ROS and protecting cells from oxidative damage. By mitigating oxidative damage, the *L. acidophilus* fermented lysate may create a more favorable environment for cell survival and tissue regeneration. Reducing the levels of ROS at the wound site can protect cells from damage, promote cell proliferation and migration, and enhance the overall healing process. The antioxidant effects of *Lactobacillus acidophilus* have been further supported by other studies. In addition to controlling negative factors that can impede healing, such as excessive inflammation and oxidative stress, components present in the *L. acidophilus* fermented lysate may also directly stimulate tissue repair processes. As previously mentioned, increased TGF-β signaling is a potential mechanism by which the *L. fermented* lysate may promote tissue regeneration. TGF-β is a growth factor that plays a crucial role in various aspects of wound healing, including fibroblast activation and collagen deposition. Fibroblasts are the cells responsible for producing the extracellular matrix components, such as collagen, which provide structural support and strength to the healing tissue. By stimulating TGF-β signaling, the *L. fermented* lysate may enhance fibroblast activity and promote the deposition of collagen, leading to improved tissue repair and remodeling. Lysates derived from bacteria, such as the *L. acidophilus* fermented lysate used in this study, contain a variety of bacterial components and metabolites that could potentially interact with host cell receptors to promote tissue repair. These components include peptidoglycans, lipoteichoic acid, and DNA fragments, as well as various metabolites. These bacterial components and metabolites may act as signaling molecules, interacting with receptors on host cells and triggering intracellular signaling pathways that promote cell proliferation, migration, and the synthesis of extracellular matrix components. While this study did not directly assess microbial counts in the wounds, the inherent antimicrobial properties of *Lactobacillus* products may also contribute to the observed enhancement of wound healing. *Lactobacillus* species are known to produce various antimicrobial compounds, such as lactic acid and bacteriocins. These compounds can inhibit the growth of pathogenic bacteria and help to maintain a lower wound bioburden. Maintaining a lower wound bioburden is crucial for preventing infection-related delays in healing. Bacterial infections can significantly impair the healing process, leading to increased inflammation, tissue damage, and delayed wound closure. By reducing the number of pathogenic bacteria in the wound, the antimicrobial properties of the *L. fermented* lysate may contribute to its superior efficacy in promoting wound healing, particularly when compared to the untreated control group. Furthermore, the potential antimicrobial effects of the *L. fermented* lysate offer an alternative mechanism to the direct bactericidal or bacteriostatic action of SSD. While SSD primarily acts by killing or inhibiting the growth of bacteria, the *L. fermented* lysate may contribute to a more favorable healing environment not only by potentially reducing the bacterial load but also by actively promoting tissue repair through immunomodulation, antioxidant activity, and direct stimulation of tissue regeneration.[14-17]

The findings of this study are consistent with a growing body of literature highlighting the therapeutic potential of topical probiotics and their derivatives in wound management. While direct comparisons between studies can be challenging due to variations in bacterial strains, preparation methods, concentrations, and wound models, the overall trend of enhanced healing is consistently observed. For instance, studies have reported positive outcomes using *Lactobacillus plantarum* supernatant in the treatment of human second-degree burns, and beneficial effects of heat-killed *Lactobacilli* in murine cutaneous wounds. These studies, along with the present research, contribute to the growing evidence

supporting the use of *Lactobacillus*-derived products as therapeutic agents for wound healing. This study specifically provides valuable data on the use of a fermented *lysate* preparation of *L. acidophilus* applied topically for the treatment of thermal burns. The use of a lysate, which is a postbiotic preparation, may offer certain safety advantages over the application of live probiotics, particularly in vulnerable patient populations such as immunocompromised individuals or in cases of extensive burn injuries. The results of this study have significant clinical implications for the management of burn wounds. The superior efficacy of the *L. fermented* lysate in promoting wound healing, as demonstrated in this research, suggests that it has the potential to be developed as a novel therapeutic agent for burn wound management. This postbiotic preparation offers a promising alternative or adjunctive therapy to current standard treatments, potentially improving clinical outcomes and reducing the morbidity associated with burn injuries. The potential advantages of the *L. fermented* lysate over traditional treatments like SSD include its multifaceted mechanism of action, which goes beyond simply controlling infection. By modulating inflammation, reducing oxidative stress, and actively promoting tissue regeneration, the *L. fermented* lysate may create a more optimal physiological environment for wound healing. This approach aligns with the growing interest in therapeutic strategies that aim to enhance the body's natural healing processes, rather than solely focusing on pathogen eradication.[18,19]

4. Conclusion

This study provides compelling evidence that the topical application of 5% fermented Lactobacillus acidophilus lysate significantly accelerates the healing of second-degree burn wounds in Wistar rats. The L. fermented lysate demonstrated superior efficacy in promoting wound closure when compared to both the standard silver sulfadiazine treatment and the untreated control group. The findings highlight the potential of this postbiotic preparation as a promising novel therapeutic agent for burn wound management. The observed accelerated healing with the L. fermented lysate is likely attributed to its multifaceted mechanism of action, which may involve immunomodulation, inflammation control, antioxidant activity, promotion of tissue regeneration, and antimicrobial effects. This study supports the growing body of evidence indicating the therapeutic potential of Lactobacillus -derived products in wound care and suggests that the L. acidophilus fermented lysate could serve as a valuable alternative or adjunctive therapy to current standard treatments, potentially improving clinical outcomes in burn wound management.

5. References

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