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RESEARCH ARTICLE
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ANALYSIS OF WOUND AREA WITH TOPICAL THERAPY OF PROBIOTICS & LOW LEVEL LASER THERAPY IN RATS

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Abstract

Background: Delayed wound healing is a major clinical concern. Low-level laser therapy (LLLT) at 660 nm and topical probiotics such as *Streptococcus thermophilus* have shown potential in accelerating tissue repair. However, comparative evidence between these modalities remains limited.

Objective: To evaluate and compare the effects of LLLT, *Streptococcus thermophilus* probiotic, and normal saline on wound healing progression over 21 days.

Methods: This experimental study included three groups receiving topical treatment: Group A (Control: normal saline), Group B (LLLT: 660 nm, 100 mW, 10 J/cm² for 30 s), and Group C (Probiotic: *Streptococcus thermophilus*). Wound samples were collected on days 3, 7, 14, and 21. Comparative analyses were performed between A & B, A & C, and B & C on selected days.

Results: Significant differences in wound healing were observed among all groups on days 3, 7, and 14. Both LLLT (Group B) and probiotic therapy (Group C) demonstrated faster wound contraction and improved tissue characteristics compared to the control. On day 21, no significant difference was found between Groups B and C, and both showed complete wound closure, whereas Group A exhibited delayed healing.

Conclusion: LLLT and *Streptococcus thermophilus* probiotic treatments significantly enhance wound healing compared to normal saline. By day 21, both modalities achieve comparable and complete wound closure, indicating their effectiveness as potential therapeutic options.

INTRODUCTION

The body's largest organ is considered the skin because of its role in regulating temperature, providing a shielding barrier mechanism, and being the most important sensory perception (1). If

someone talks about its structure, then it consists of a few layers like the epidermis, the middle layer is the dermis, and lower lowermost layer is subcutaneous tissue, but all layers perform their crucial role in the maintenance of homeostasis and ultimately in the healing of wounds (2). After getting injured, the integumentary system starts the various processes of healing, including a variety of inflammatory mechanisms, proliferative, and remodeling mechanisms (3). Conversely, in diabetic patients, this mechanism is highly compromised, resulting in chronic infectious wounds and less healing (4). In diabetic patients, due to raised blood sugars, collagen formation is compromised, and dysregulation of blood vessel formation is noticed (5). Reactive oxygen species also play their role in delayed wound healing (6). That's why multiple strategies for wound treatment in diabetic patients are needed. Low-Level Laser Therapy (LLLT) is a technique in which a variety of wavelengths are utilized for healing purposes (7). Its role in wounds is noted as a lightning pain agent and speedy healing (8). Synthesis of collagen & production of fibroblasts are also noticed (9).
Another agent known as probiotics has added consideration for its positive effect on the healing process (10). Amongst a variety of strains, the *Streptococcus thermophilus* (ST) has a barrier function because of the production of ceramide and controlling the process of hydration, hence relieving the inflammation successfully (11). The antimicrobial function was also noticed in diabetic wounds (12). *S.T* has a good role in the healing of wounds because of the regeneration of epithelium (13)
Probiotics and LLLT provide a beneficial role, but in diabetic wounds, further research is needed to explore which one is better.

MATERIAL & METHODS

Study Design and Setting of the Study

The study was experimental. The animals used in this study were Wistar Albino rats obtained from the animal house of the Isra University Karachi campus. This study was conducted from January 2021 to August 2021.

The study involved 18 rats, and the sample size was calculated using the "E" formula, where:
E=Total number of animals−Total number of groups
The study included two experimental groups and one control group, with each group comprising six animals.

Selection of Animals and their Grouping

Approval for this study was obtained from the Institutional Ethical Committee of Isra University Karachi campus before starting the study. The animals were male rats, and their weight was **150-250 g,** selected randomly. After the selection division is done into the following groups:

- **Group A (Control):** No treatment (application of only normal saline on the wound area superficially (topically).
- **Group B (LLLT):** Application of Low-Level Laser Therapy on the wound area superficially (topically) once daily. **The wavelength used is 660nm, 100mw power with 10 j/cm^2 for 30 sec.**
- **Group C (Probiotic Treatment):** Application of *Streptococcus thermophilus* on the wound area superficially (topically) once daily. (**1ml solution had 10^{10} to 10^{11} CFU/ml of bacteria)**

The Process of Wound Formation and Its Measurement:

We anesthetized all animals with ethanol. After completion of the shaving and cleaning process, A **cutaneous wound of (1.5 × 1.5 cm²)** size was taken. It was a **full-thickness standard size** created with the help of a sterile scalpel. The measurement of the area of the wound was done with the help of a measuring scale on different days (3, 7,14, and 21st day). A DSLR camera was used for Photographs.

Statistic methods

SPSS software was utilized for the purpose of analysis. The version was 23.0, and the test applied to compare the grouping system was ANOVA & Post-hoc Tukey test. The mean ± S.D is used for the expression of values with $p \leq 0.05$.

Results:

Comparison of Wound size (cm2) between Group A and B

Mean ± SD of wound size in groups A(1) were 1.8625 ±0.4432, A(2) 1.7688 ± 0.07039S, A(3) 1.4625 ± 0.10607 and A(4) 1.2125 ± 0.14577 cm2 and B(1) were 1.5375 ± 0.10607, B(2) 1.1125 ± 0.11260, B(3) 0.2500 ± 0.20702 and B(4) 0.0000 ± 0.00000.

The difference between group A(control) & B (laser) on day 3 (A1), day 7 (A2), day 14 (A3), Day 21 (A4), with day 3 (B1), day 7 (B2), day 14 (B3), Day 21 (B4) was significant with P = 0.002, P=0.001, P=0.001, P=0.001 respectively mentioned in figure I & II.

Comparison of Wound size (cm2) between Groups A and C

Mean ± SD of wound size in groups A(1) were 1.8625 ±0.4432 A(2) 1.7688 ± 0.07039, A(3) 1.4625 ± 0.10607 and A(4) 1.2125 ± 0.14577 cm2. In C(1) were 1.4250 ± 0.26049, C(2) 1.1750 ± 0.10351, C(3) 0.9000 ± 0.10690 and C(4) 0.0000 ± 0.00000.

The difference between group A(control) & C(ST) on day 3 (A1), day 7 (A2), day 14 (A3), Day 21 (A4), with day 3 (C1), day 7 (C2), day 14 (C3), Day 21 (C4) was significant with P = 0.002, P=0.001, P=0.001, P=0.001 respectively mentioned in figure I & II.

Comparison of Wound size (cm2) between Groups (B) and (C)

Mean ± SD of wound size in group B(1) were 1.2650 ± 0.4721, B(2) 0.8838 ± 0.7308, B(3) 0.2500 ± 0.20702 and B(4) 0.0000 ± 0.00000. Mean ± SD in C(1) were 1.4250 ± 0.26049, C(2) 1.1750 ± 0.10351, C(3) 0.9000 ± 0.10690 and C(4) 0.0000 ± 0.00000.

The difference between group B(Laser) & C(ST) on day 3 (B1), day 7 (B2), day 14 (B3), with day 3 (C1), day 7 (C2), day 14 (C3) was significant with P = 0.003, P=0.002, P=0.002 respectively. The wound contraction was noticed on these days early in the laser group. But ultimately, complete closure of the wound was noticed on day 21, so there was no difference on this day. It's mentioned in Figures I & II.

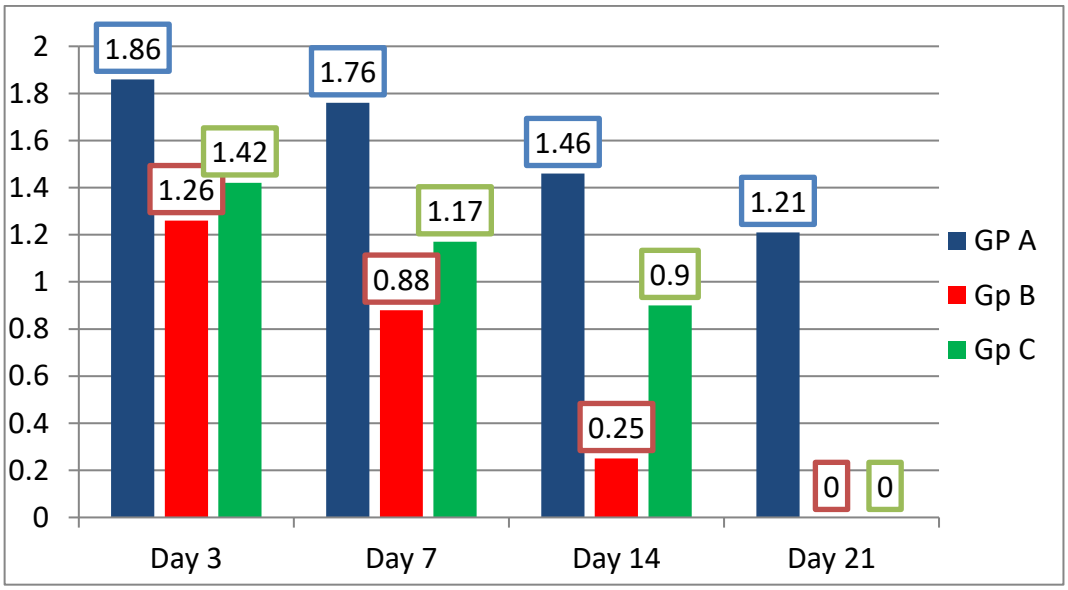

Figure I: (Bar Chart)

This shows a Comparison of the mean size of wounds in different groups

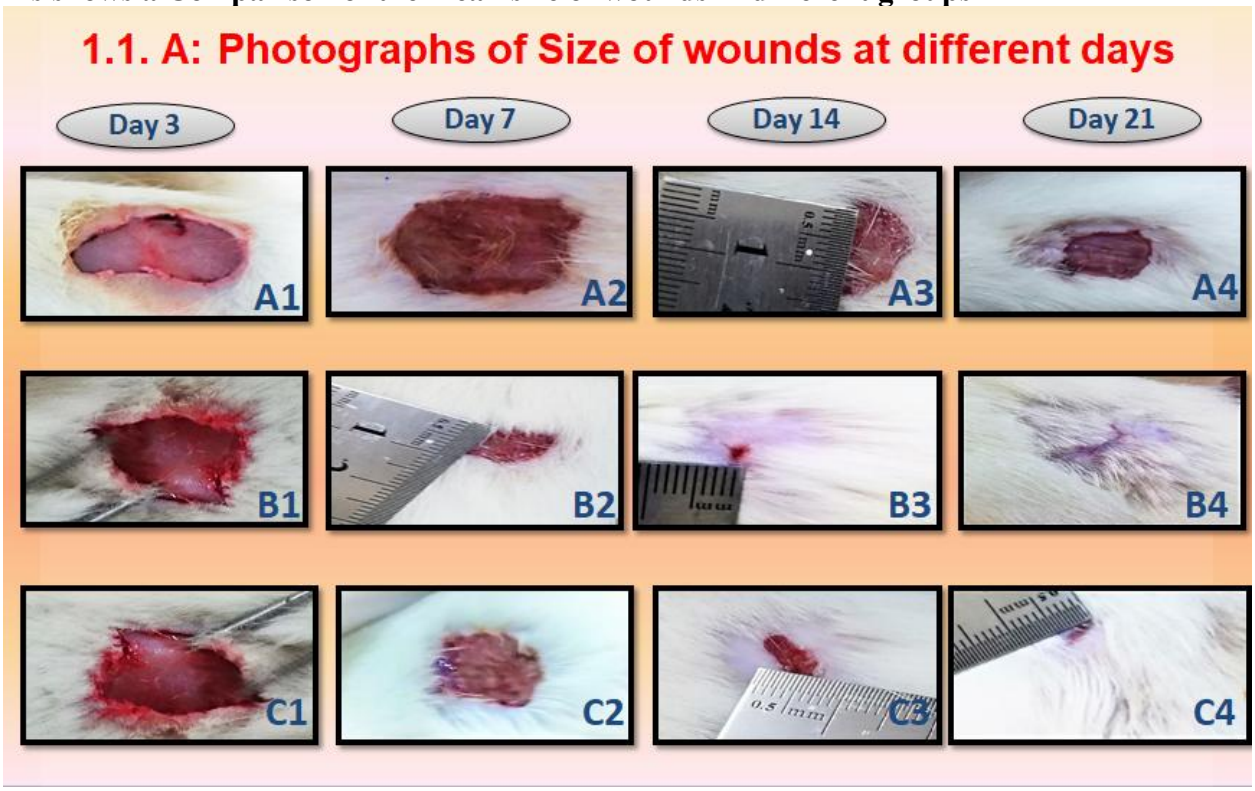

Figure II: Photograph showing the Size of the wound on different days of the experiment in groups A, B, & C.

Discussion

In the current study, we evaluate the size of wound reduction amongst the different groups. We observed that the control group, which is group (A), the low-level laser therapy (LLLT) group, which is group (B), and group (C), the Streptococcus thermophilus Group B, exhibited the maximum & consistent reduction in the size of the wound. However, on the last day, there was no statistically significant alterations were noticed amongst Group B and Group C, indicating that S. thermophilus topical applications may accomplish similar outcomes but slightly delayed.

One of the trials based on the clinical approach by Özer and İnci in 2024 found that LLLT has a perfect effect on the healing of wounds done after the extraction of teeth (14). Parmadiati et al. in 2024 declared that LLLT is very potent in the formation of re-epithelialization, hence helping in closing wounds rapidly(15).

In this experimental study, we observed that in the LLT-treated group, wound healing was faster compared with the control group and the Streptococcus thermophilus

S. thermophilus has arisen as a topical treatment in dermatology. It is because of ceramide creation and barrier strengthening. In the current study, Group C presents with progressive contraction of wound size, ultimately accomplishing the same consequences as LLLT by the end day. Another study was done in 2022 by Lombardi et al., in which it was noticed that the fibrotic signaling pathways were inhibited by S. thermophilus lysate. The TGF-β1/Smad, in the dermal fibroblasts, signifies its controlling effect in the formation of scar and repairment of the tissue (17). These outcomes offer a reasonable clarification for the lessening wound size seen in Group C.

Another study showed that low-level laser therapy enhances the healing process in dermal abrasion in Adult male BALB/c mice because low-level laser therapy promotes collagen synthesis, reduces the wound area rapidly, and helps in complete re-epithelization in skin. The same thing was observed in the current study that synthesis of collagen fibers was excellent in laser laser-treated group when compared with those rats treated with normal saline. The better results of re-epithelization and thickness of the dermis and epidermis were observed in laser laser-treated group when compared with those rats treated with normal saline. (18).

In our experiment, we observed that both the laser and Streptococcus thermophilus-treated groups showed wound healing and formation of epidermis when compared with the normal saline-treated group. However, the laser-treated group showed early healing of wounds with a better thickness of epidermis compared with the streptococcus thermophilus group.

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