Original Article

Anti-Inflammatory Role of Low Level Laser Therapy and Zinc Oxide in Wound Repair: A Comparative Study in Rats

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ABSTRACT

Objective: Wound healing is a widely studied subject, and efforts are being made worldwide to make wound healing more effective. We carried out a study to assess the anti-inflammatory role of low level laser therapy and zinc oxide in wound repair.

Study Design: Random sampling technique study

Place and Duration of Study: This study was conducted at the Karachi Campus of Isra University. The study was carried out for duration of 6 months, from June 2020 till December 2020.

Materials and Methods: 18 Albino rats were selected through random sampling and divided into three groups. Each group was given a normal daily diet along. An equal incision was made into all the rats of the group of the same size. Intervention was then carried out in which Group A received topical saline solution hence acting as the control group, Group B was given Low Level Laser Therapy (LLLT), and Group C was given Zinc Oxide topically. Histological tissue was attained on the 3rd and 7th day for analysis. Data was analyzed using SPSS with one-way ANOVA followed by post-Hoc tukeys test being applied.

Results: Reduction in the Neutrophil and Lymphocyte count was seen in Group B (Day 3: 19.65±2.30, Day 7: 8.55±5.1) and Group C (Day 3:23.00±8.30, Day 7: 18.44±3.34), however Group B in which LLLT was the interventional agent demonstrated the most favorable result. Significant difference was obtained when comparing group B to A on day 3 (<0.01) and 7 (<0.001), as well as when Group B was compared to Group A on day 3 (0.01) and day 7 (<0.001).

Conclusion: Our study concludes that both LLLT and Zinc Oxide improve wound healing through their anti-inflammatory actions; however, LLLT showed more favorable results.

Key Words: Anti-Inflammatory Role, Level Laser Therapy, Zinc Oxide, Wound Repair

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INTRODUCTION

The Human body is a magnificent specimen which we have gone onto study for centuries now yet have encountered hindrance in fully understanding each and every part of it and finding solutions that can prolong the human life. The body derived of many organs, is covered by the skin which is the largest organ serving as a covering to our nerves, vessels, muscles, and other organs.

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Like all other cells in our body, the skin constantly renews and multiplies itself thereby staying relevant and performing its physiological function to the maximum capacity. Furthermore, if the skin gets damaged in any way, it too heals like the rest of the body. This process is known as wound healing which is a process by which the skin attempts to heal itself. The mechanism of wound healing has been recognized as important to health since the beginning of mankind[1]. Wound healing is a complex reaction which can be divided into 3 phases, inflammatory phase, proliferative phase, and the remodeling phase. A haemostatic phase occurs just before the inflammatory phase, in which platelet aggregation and clot formation occurs to prevent further bleeding. Inflammatory phase is characterized by in which neutrophils, lymphocytes, and macrophages are recruited and arrive to the site of wound to remove any microorganism that can utter harm to the body[2-3]. Proliferation phase begins next in which production of collagen, ground substance, angiogenesis, and re-epithelialization of the wound takes place[4-6].This is what is called the formation of

granulation tissue. Finally, the maturation phase is the last phase of wound healing in which maturation and remodeling of collagen and closure of the wound takes place[7-8]. Wound healing isn't always a successful process and can lead to complications such as delayed wound healing, hypertrophic scar, keloids, and even infection of the wound[9-11]. Another consequence of improper wound healing is the formation of a scar. Evidence is present which suggest that the main culprit for scar formation is due to prolong inflammatory phase during the process of wound healing[12-13]. Many attempts have been made to improve wound healing by reducing the inflammatory phase of wound healing. This can be done by substances or materials that have anti-inflammatory properties. Low Level Laser therapy (LLLT) has come out as a strong candidate in promoting wound healing due to its anti-inflammatory action[14]. Similarly, Zinc oxide is also taken into consideration due to its properties[15]. Wound healing is a major cause of concern worldwide and studies are continuously ongoing to find ways to make wound healing as quick and efficient as possible without any complications. Considering this, a study was conducted to assess the anti-Inflammatory role of LLLT and zinc oxide in wound repair.

MATERIALS AND METHODS

After taken the necessary steps in attaining approval from the Institutional review board (IRB), an interventional study was conducted at the Karachi campus of Isra University. The study was carried out for duration of 6 months, from June 2020 till December 2020. 18 albino rats were selected on the basis of random sampling technique. These rats were acquired through the animal house of Al-Tibri Medical College. Each albino rat that was included in the study needed to weight 150-250gms which was done through an electronic weigh scale. The rats were then divided into groups of three, with each group consisting of six rats. The following parameters were set for each group

Group A: Control Group received normal saline (0.9%) solution topically once daily for 7 days.

Group B: Therapeutic Group that received Low Level Laser Therapy 4j/cm^2 for once daily for 30 seconds for 7 days.

Group C: Therapeutic Group that received 20% Zinc Oxide solution once daily for 7 days.

Before performing the intervention, the wound was surgically created in the dorsal area of their back. Anesthesia was given in the form of chloroform and their backs were shaved and clean before a sharp sterilized scalpel was used to make a cutaneous wound that had a dimension of 1.5x1.5cm^2. Confirmation of the measurement of the incision was done using a plastic scale. All rats had a normal daily diet throughout the study.

To analyze the histology of the tissue, sample was taken on the 3rd and 7th day. The tissue extracted from the wound site was fixed using formalin solution, after which it was sectioned using a microtome. Further processing was done using the H&E staining. The slides were examined under a light microscope at magnification of 100x and 400x to count the number of inflammatory cells via reticule count, with a total five number of boxes being taken into account for the cell count. The number of neutrophils and lymphocytes were identified and then counted.

Data was then analyzed using SPSS Version 20.0. Data analysis was done using one-way ANOVA test followed with post-Hoc tukeys test. A p value of ≤0.05 was considered to be significant**.**

RESULTS

Neutrophil Count

Group B and A: Mean ± SD of neutrophil count at 400x magnification on day 3 in Group B was 19.65±2.30 and in Group A was 39.85±6.40. Significant difference was seen in Group B compared to Group A (p<0.01). Mean±SD of neutrophil count at 400x magnification on day 7 in group B was 8.55±5.1 and in group A was 33.36±4.4. Significant difference was seen in Group B compared to Group A (P<0.01). (Table 1) (Figure 1)

Group B and C: Mean±SD of neutrophil count at 400x magnification on day 3 in Group B was 19.65±2.30 and in group C was 23.00±8.30. Significant difference was seen in Group B compared to Group C (p<0.01). Mean±SD of neutrophil count at 400x magnification on day 7 in group B was 8.55±5.1 and in Group C was 18.44±3.34. Significant difference was seen in Group B compared to Group C (p<0.01). (Table 1) (Figure 1).

Table No.1: Level of Significance when comparing Neutrophil Count between Groups on Day 3 and 7

Comparison of Groups	Day 3 level of significance	Day 7 Level of Significance
B vs A	<0.01	<0.001
B vs C	<0.01	<0.001

P value≤0.05

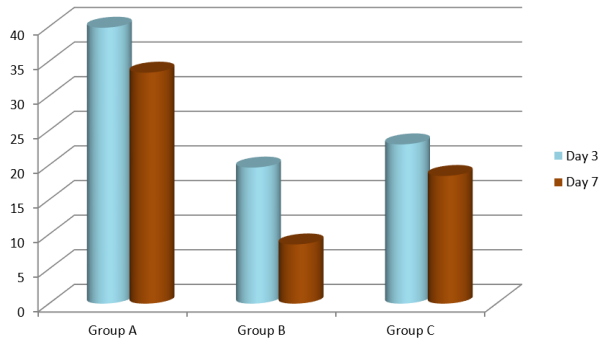

Figure No.1: Number of Neutrophil Count in each Group on 3rd and 7th Day

Lymphocyte Count:

Group B and A: Mean ± SD of Lymphocyte count at 400x magnification on day 3 in Group B was 69.73±1.20 and in Group A was 33.59±2.65. Significant difference was seen in Group B compared to Group A ($p<0.001$). Lymphocyte count on day 7 in Group B was 38.34±1.03 and in Group A was 54.73±1.53. Significant difference was seen in Group B compared to Group A ($p<0.001$). (Table 2) (Figure 2)

Group B and C: Mean±SD of Lymphocyte count at 400x magnification on day 3 in Group B was 69.73±1.20 and in Group C was 49.82±0.92. Significant difference was seen in Group B compared to Group C ($p<0.001$). Lymphocyte count on day 7 in Group B was 38.34±1.03 and in Group C Was 40.45±0.95. Significant difference was seen in Group B compared to Group C ($p<0.001$). (Table 2) (Figure 2).

Table No.2: Level of Significance when comparing Lymphocyte Count between Groups on Day 3 and 7

Comparison of Groups	Day 3 level of significance	Day 7 Level of Significance
B vs A	<0.01	<0.001
B vs C	<0.01	<0.001

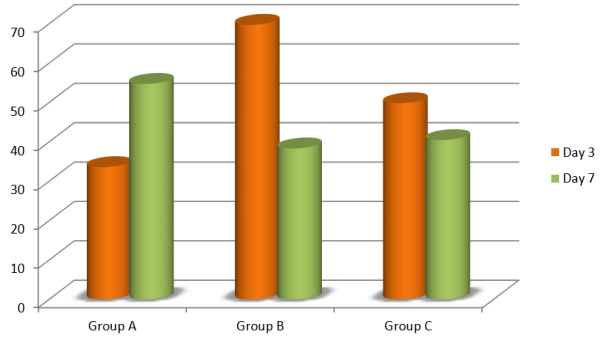

Figure No2: Number of Lymphocyte Count in each Group on 3rd and 7th Day

DISCUSSION

Wound healing if done without any complications, can lead to the formation of a perfectly normal and healthy skin. However, this is not always seen and researchers have worked tirelessly to find ways as to improve wound healing. LLLT seems to be a good candidate. Our study has shown that LLLT can actually reduce the number of neutrophil and lymphocyte count in wounded tissues. LLLT indeed does show significant increase in skin as well as scar tissue recovery, through the presence of anti-inflammatory properties[16]. Another study also showed that LLLT can reduce the levels of pro-inflammatory cytokines and increase the levels of anti-inflammatory cytokines thereby reduces the inflammation cells and inflammation period. This in turn helps to increase collage fibers and form a well-made granulation tissue component in the wound[17]. Another study conducted by Franciane et al (2014), showed that LLLT-treated groups demonstrated decrease in the inflammatory cells number, while simultaneously increasing collagen deposition. He showed that irradiation by laser at 3 and 4 J/cm^2 caused a reduction in the inflammatory process; these findings are in line with our study in which there is also a reduction in the inflammatory cell count[18]. Zinc Oxide was another substance part of our study and also went onto show commendable results by reducing the number of white blood cells in the wound tissue. Zinc Oxide is used in modern medicine for its many properties such as antimicrobial and wound healing[19]. In our study zinc oxide promoted wound healing by reducing both the neutrophil and lymphocyte count. In another study conducted by Cangul et al (2006) also showed the zinc oxide reduced inflammatory cell count, however, in that particular study it was compared to topical tripeptide-copper complex which reduced the inflammatory cell count more than zinc oxide[20]. Similar results can be highlighted in our study. Both LLLT and zinc oxide successfully promoted wound healing by promoting anti-inflammatory activity which can be evident by seeing the reduction in neutrophil and lymphocyte count on the respective days, but LLLT came out to be the superior agent when both of the two study groups were compared. There are many other anti-inflammatory agents that promote wound healing which can be further studied in the future and compared with our parameters[21-24]. Wound development and its healing is inevitable, therefore we must be prepared to tackle it in a way which make the process as smooth as possible by reducing the inflammatory phase and forming near perfect skin or a favorable scar tissue.

CONCLUSION

Low level laser therapy and zinc oxide successfully managed to lower the neutrophil and lymphocyte count during wound healing, this was done by their anti-inflammatory properties. However, LLLT group was more effective in reducing the inflammatory cells.

Author's Contribution:

Concept & Design of Study:	Jawad Mumtaz Sodhar
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Final Approval of version:	Jawad Mumtaz Sodhar

Conflict of Interest: The study has no conflict of interest to declare by any author.

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