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Original Research Article

Anti-inflammatory effects of topical lactobacillus acidophilus and antibiotic in wound repair on the basis of microscopic parameters: a comparative study in rats

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ABSTRACT

Background: Wound treatment still a task for medical professionals, according to the time advancement. We need to elaborate further new interventions to cope up this common factor of community. The objective of the study was to assess the anti-inflammatory topical role of Lactobacillus acidophilus with antibiotic in wound repair of rats based on of microscopic parameters. This comparative study was conducted in the Department of Anatomy Al-Tibri Medical Collage and Hospital Isra University Karachi Campus from December 2018 to April 2019.
Methods: Total 18 male of wistar rats were randomly selected for this study. The study comprised of three topical groups Control, Antibiotic and Lactobacillus acidophilus groups respectively.
Results: The samples were taken from the wound site for the preparation of microscopic slides, to count the numbers of neutrophils, lymphocytes and macrophages for the evaluation of anti-inflammatory process in both groups on day 3 and 7. Data obtained were analyzed by SPSS version 20 by applying One Way ANOVA and post hoc Tukey's test.
Conclusions: The results were shown potent anti-inflammatory effects of *Lactobacillus acidophilus* group in comparison with other conventional therapy. The study concludes that the topical application of *Lactobacillus acidophilus* had remarkable anti-inflammatory effects in wound management.

Keywords: Albino rats, Anti-inflammatory, Lactobacillus acidophilus Antibiotic, Wound

INTRODUCTION

Even with substantial progresses in operative precaution, surgical site infections (SSIs) still a main factors contributing to death. Involvement of a healthy microbiome in the pre-surgical phase may assist regulator of multi-drug resistance (MDR) bacteria. A loss of microbial abundance can delay the wound healing. The pro-biotics are used to cover the infection that has been assessed by means of multiple studies, but their efficacy is questionable up till now.[1] Bacterial load is supposed to show a substantial part in delay wound repair of chronic wounds and the growth of infection based problems.[2] Dermal tissues are mainly full of microbiota. Therapeutic diversity may change the biochemical composition of tissue. The effect of agents that might triggers the host–microbe contacts of the skin. Impeding a vital factor of

the balance cascade reduced diversity and transformed the composition of the dermal tissue microbiota, similar to a drop in skin inflammatory cell penetration of skin resistance and immune gene expression. These results suggest an interactive role between complement and the microbial ecosystem of the skin and could have significant inferences for inflammatory skin disorders.[3] Kefir is a common form of dairy product that is composed of particular compounds of bacteria.

Fermented yogurt showed various beneficial effects on dermal health. Probiotic showed remarkable effects on multiple system of the body like digestion, allergic reactions, renal functions and also in maternal and fetal wellbeing.[4] The use of probiotics have proven their safety profile, mainly it shows a tremendous role in the treatment of various skin related incidence.[5] Another mechanism involved in multiple dermal allergic responses is dysfunction of dermal barrier and its leads to create a weaknesses specially in cases of Atopic dermatitis, also seem to disrupt the intestinal mucosa and the configuration of its microflora is altered in numerous allergic patients. Some of the cases suffering from Atopic dermatitis the wall are weakened, in the dermal as well as in the mucosal lining of intestine, producing the transmission of antigens. In fact in such cases the *Lactobacillus* was found to boost up the barrier purpose for restoration. The consumption of probiotics has been investigated for the managing of numerous infections, related with dermatology. Yet the outcome of studies is debatable. They are live microorganisms with immunomodulatory properties and have a positive effect on the hosts' health by altering immune reaction, opposing with destructive gut flora, toxins, and host materials, thus enhancing the gut barrier action.[5] The common species are *lactobacillus acidophilus* and *enterococci*, and each of them are strong immunomodulator, generating pro and anti-inflammatory cytokines. Probiotics can stimulate the Th1 cytokines and intern reduces the Th2 response that controls the immune response and represent by showing an in reverse levels of immunoglobulin E (IgE), eosinophilia, and interferon-gamma (IFN-γ) 6, subsequently in that way they reduces the inflammatory path. They prove the capability to speed up the recovery of skin barrier function[6].

METHODS

An experimental study was conducted at Al-Tibri Medical College and Hospital Karachi for the duration of October 2018 to April 2019. Total 18 numbers of rats were included in this study. The sample size was calculated by using formula of "E".[7]

The value of "E" lies between 10 and 20.
E= Total numbers of animals- total numbers of groups
(One group is control and other 3 experimental groups with 7 numbers of rats/group)
E= (7x3)-3
E= 21-3
E= 18 (six numbers of rats/group).

Sampling

It was done after the approval of Institutional ethical committee and the animal were purchased from the animal house of Al-Tibri Medical College and Hospital Karachi. Animal with the weight between 150-250 gms, and randomly included in this study and diseased animals were excluded from the study. The animals were separated into three groups on the topical therapeutic agents, each one comprises of 6 animals in each group. Group A (control). Group B (Probiotics), Group C (antibiotic).

Treatment plan

Topical application of normal saline in control group A, liquid form of *lactobacillus acidophilus* in group B and Neomycin cream in group C once daily for 21 days. *Lactobacillus acidophilus* was isolated from yogurt, which was purchasing from the market. They were isolated from the lab of microbiology of PCSIR research center in Karachi. They were isolated on agar MRS agar medium, and certificate of authentication of specie was given from the microbiology department.

Wound formation

All animals were anesthetized was given chloroform in close jar for few minutes, then their dorsal area of back were shaved and clean. With the help of sharp scalper the cutaneous wound were formed with the measurement of 1.5x1.5cm^2. The area was measured by a plastic scale. All animals were kept on normal daily diet and were isolated in different cages after tagging them. The sample was taking from the cutaneous tissue of skin on day 3 and 7 for their histological analysis.

Slide preparation

For the purpose of histological examination of tissue, the sample was taken from the margin of the wound on day 3 and day 7 from all experimental groups. After extraction the tissue was kept in formalin for the fixation. Sectioning was done through microtome, and then further processing of slide preparations and staining with Heamatoxilin and Eosin.

Microscopic examination

The slides of skin were examine under the light microscope 100x and 400x for the counting of inflammatory cells through reticule count, total five numbers of boxes were taken in account for the cell count. Total numbers of neutrophil, lymphocytes and macrophages were identified and count in square field area of reticule. The images were taken through DSLR camera.

Statistical analysis

The data was analyzed by using SPSS version 20.00. All the data were enter on excel sheet, then they were analyzed by using one way ANOVA test followed by post Hoc tukeys test. The p value was considered to be significant with confidence interval of 0.05 and p value was ≤0.05.

RESULTS

Number of neutrophils count

Group B and A

Mean±SD of numbers of neutrophils/x400 in group B on day 3 was 18.25±2.28 and in group A were 37.75±6.33. The significant value was in group B as compared to group A (P<0.001).

In group B on day 7 was 9.45±5.0698 and in group A was 31.75±4.2560 with significant value was in group B as compared to group A on day 7 was (P<0.001) so, decrease number of neutrophils in Group B as compared to Group A as they are in (Figure 1) and (Figure 1.1, 2.1 and Table 1).

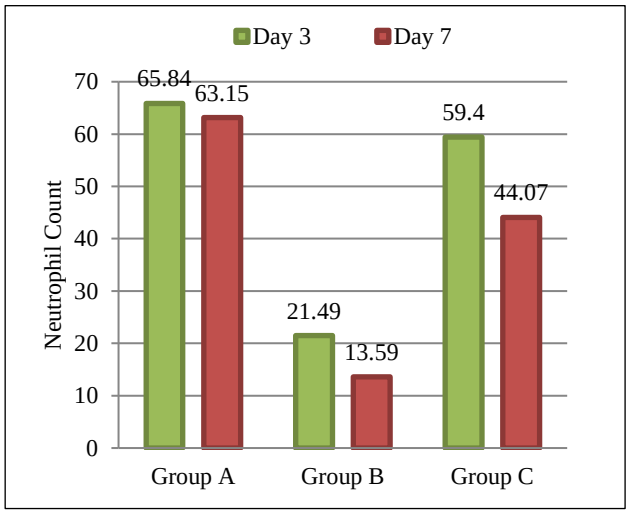

Figure 1: Comparison of Mean ± SD of neutrophil count in group A B and C on day 3 and 7.the numbers of neutrophil showing significant decrease in group B that shows the anti-inflammatory effect of probiotic in wound repair.

Table 1: level of significance in comparison of Neutrophil count between the groups.

Comparison between the groups	Level of significance On Day 3	Level of significance On Day 7
B vs A	<0.01	<0.001
B vs C	<0.01	<0.001

Significant P value <0.05.

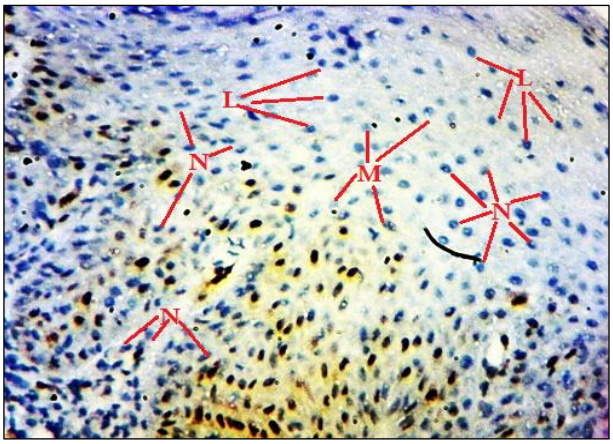

Figure 1.1: Dermal tissue (H&E stain) of Group A an increased numbers of neutrophil count (N), decreased numbers of lymphocytes (L) and decreased numbers of macrophage (M) at x400.

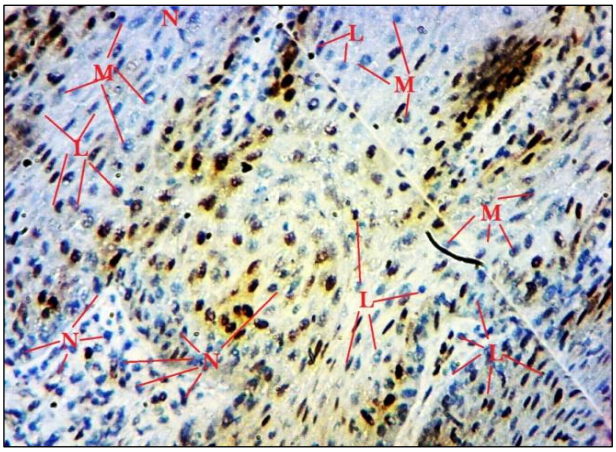

Figure 2.1: Dermal tissue (H&E stain) of Group B decreased numbers of neutrophil counts (N), increased numbers of lymphocytes (L) and increased numbers of macrophage (M) at x400.

Group B and C

Mean±SD of numbers of neutrophils/x400 in group B on day 3 was 8.25000±2.2836 and in group C were 24.0000±6.1250. The significant value was in group B as compared to group C (P<0.001), In group B on day 7 was 5.4590±5.0698 and in group C was 19.7500±3.2560 with significant value was in group B as compared to group C on day 7 was (P<0.001) so, that shows the decrease number of neutrophils in Group B as compared to Group C, as they are in (Figure 1) and (Figure 2.1, 3.1 and Table 1).

Number of Lymphocytes

Group B and A

Mean±SD of numbers of lymphocytes/x400 in group B on day 3 was 67.91±1.07 and in group A were 34.43 ± 2.35. The significant value was in group B as compared to group A (P<0.001). In group B on day 7 were 39.20 ±

1.04 and in group A were 52.76±1.63 with the significant value (P<0.001), that shows the increase number of lymphocytes in Group B as compared to Group A on day 3 and 7. As they are in (Figure 2) and Figure (1.1, 2.1) and Table 2.

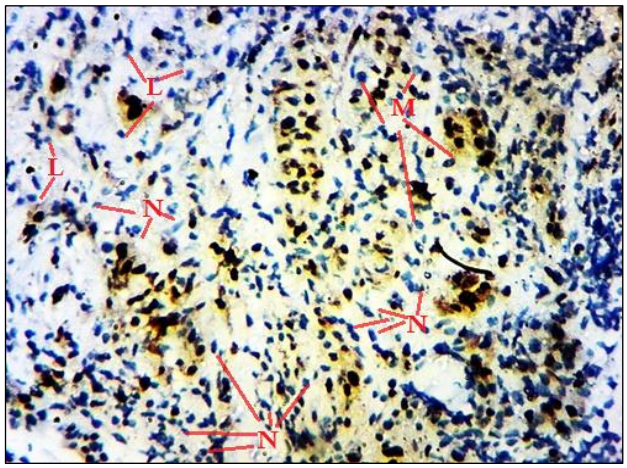

Figure 3.1: Dermal tissue (H&E stain) of Group C an increased numbers of neutrophil counts (N), decreased numbers of lymphocytes (L) and decreased numbers of macrophage (M) at x400.

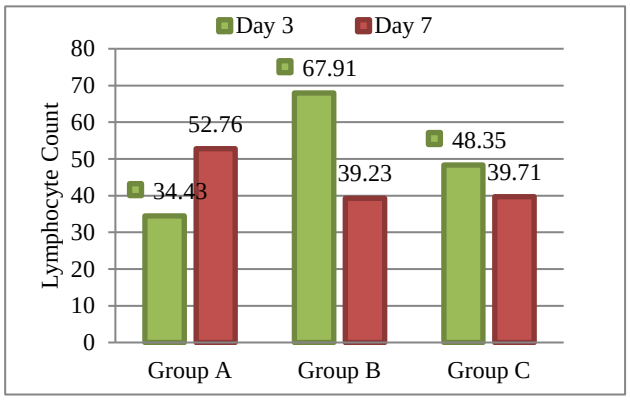

Figure 2: Comparison of Mean±SD of lymphocytes count in group A B and C on day 3 and 7.the numbers of lymphocytes showing significant increase in group B that shows the anti-inflammatory effect of probiotic in wound repair.

Table 2: Level of significance in comparison of Lymphocyte count between the groups.

Comparison between the groups	Level of significance On Day 3	Level of significance On Day 7
B vs A	<0.01	<0.001
B vs C	<0.01	<0.001

Significant P value <0.05.

Group B and C

Mean±SD of numbers of Lymphocytes/x400 in group B on day 3 was 67.91±1.07 and in group C were 48.32±0.91. The significant value was in group B as compared to group C (P=0.00). In group B on day 7 were 39.20±1.04 and in group C were 39.71±0.94 with significant value (P<0.001) that shows the increase number of lymphocytes in Group B as compared to Group C on day 3 and 7. As in (Figure 2) and (Figure 2.1, 3.1 and Table 2).

Number of Macrophages

Group B and A

Mean±SD of numbers of macrophage/x400 in group B on day 3 was 20.61 ± 0.76 and in group A were 10.13 ± 0.67. The significant value was in group B as compared to group A (P<0.001), on day 7 in group B was 15.57 ± 0.80 and in group A was 8.95±1.3459. The significant value was in B as compared to group A (P<0.001), that shows the increase number of macrophages in Group B as compared to Group A on day 3 and 7, as in (Figure 3) and (Figure 1.1, 2.1 and Table 3).

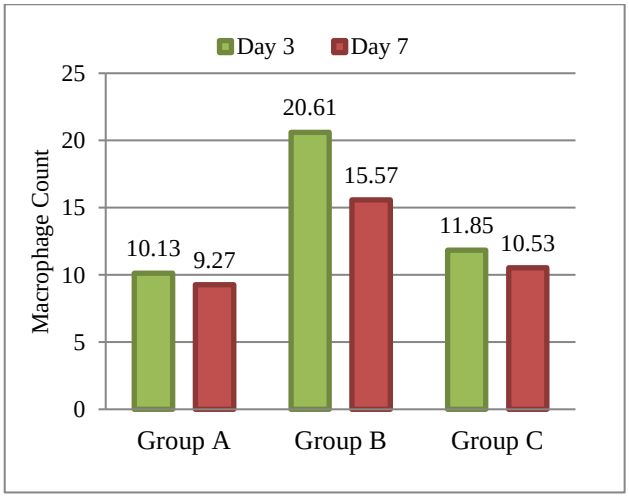

Figure: 3 Comparison of Mean±SD of macrophage count in group A B and C on day 3 and 7.the numbers of macrophage showing significant increase in group B that shows the anti-inflammatory effect of probiotic in wound repair.

Table 3: Level of significance in comparison of Macrophage count between the groups.

Comparison between the groups	Level of significance On Day 3	Level of significance On Day 7
B vs A	<0.01	<0.001
B vs C	<0.01	<0.001

Significant P value <0.05.

Group B and C

Mean±SD of numbers of macrophage/x400 in group B on day 3 was 20.61±0.762 and in group C were 11.85±1.11. The significant value was in group B as compared to group C (P<0.001), on day 7 in group B was 15.57±0.80 and in group C was 10.53±0.65. The significant value

was in B as compared to group C (P<0.001), that shows the increase number of macrophages in Group B as compared to Group C on day 3 and 7, as in (Figure 3) and (Figure 2.1, 3.1 and Table 3).

DISCUSSION

A huge range of topical therapeutics that is available for the treatment of wound repair. Probiotics are the strong source for the management of wound repair either wound related to surgical, mechanical, open or closed wound. The evidences suggest the potent effects of probiotics in the executive treatment of wound. In this study the role of probiotic showed as a strong healer in comparison to other conventional treatment.

The activation of gut-based bacteria is vital for the stimulation of regulatory cells. Intestinal colonization by *Lactobacillus* and other species, that is predictable in enhancing the immune response and gradually suppressing the pro-inflammatory cytokines activity, decreasing the progress of pre-cancerous lesions related to colon and inflammation of the mucosal lining in different inflammatory conditions.[8]

Most widely used bacteria's are lactobacillus bifidobacterium and enterococci, are specified immunomodulatory agents, by generating pro and anti-inflammatory cytokines similar in this study they showed anti-inflammatory effects.[9]

Probiotics facilitates Th1 cytokines and decreases the Th2 reaction. Having an act on Tregs, that will controls the immune response, showing an in reverse associated level with immunoglobulin E (IgE), eosinophilia, and interferon-gamma (IFN-γ) 6, hence Tregs reduces the inflammatory path. Meanwhile furthermore proven the capability to speed up the recovery of the skin barrier function as shown in this study the fastest recovery by *lactobacillus* species.[10]

Existing medical managements can be expensive and time intense. In this study the three CAMP therapies were used for wound repair and one of the therapeutic agents was probiotic that showed speedy recovery of wound with short duration of hospitalization and cost effective as similar in our study the probiotic accelerates the fastest healing process.[11] Probiotics are valuable microorganisms, had a progressive effect on human health, chiefly in fight in contradiction of pathogens. Probiotics have been connected with boosted healing of intestinal related ulcers, and infected cutaneous wounds. The modern results related to probiotics on gut epithelium and skin.[12,13] Established mechanisms by which probiotic bacteria exert their beneficial effects include direct killing of pathogens, viable translation of pathogenic bacteria, strengthening of epithelial barrier, stimulation of fibroblasts, and epithelial cells' movement and their actions. Valuable immune responses of probiotics are modulation and initiation of lymphocytes aggregation, macrophages, through prompted production of cytokines. Systemic properties of positive bacteria and connection between gut microbiota, immune system and cutaneous health through gut–brain–skin axes. In light of growing antibiotic resistance of pathogens.[14,15]

Throughout the process of wound healing, cells (macrophages, mast cells, or damaged fibroblasts) are gathered in effective tissues, which encouraged the discharge of proinflammatory mediators, therefore the amount of factors like TNF-α, IL-1β, as per study results that shown the availability of lymphocytes and macrophages at the wound site earlier to boost up the immune response.[16,17]

CONCLUSION

It is concluded that the topical application of Lactobacillus Acidophillus group showing remarkable healing effects as to the conventional therapy. It also revealed the potent anti-inflammatory effects of probiotic that enhance the repairing of wound.

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Conflict of interest: There is no conflict of interest
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