

Comparative Study of Cutaneous Wound Repair Observed in Probiotic in Relative To Antibiotic Therapy in Rat Model

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ABSTRACT

Objective: To observed the substantial evidence regarding the significance of probiotics in cutaneous wound repair in comparison with antibiotic treated group in rat model.

Place & Duration: Experimental study, conducted in the department of Anatomy, Al-Tibri Medical College Isra University Karachi Campus. From June 2013 to October 2013.

Material & Methods: Sixty Albino rats were taken and divided in to three groups on the bases of topical applications, Group A control (normal saline), Group B (Probiotics) and Group C (Antibiotic). Each group is further divided into sub group on the bases of day of sampling, A1 (day 03), A2 (day 07), A3 (day 14) and A4 (day 21). Sample was taken on day 3, 7, 14 and 21 from each group and tissues were processing and stained to observed the neutrophil count, that shows the rapid phase of healing process. Data were analyzed on SPSS version 17 by applying ANOVA post hoc Tuckey's test.

Results: The mean \pm SD of the neutrophil count of Group A (Control) and Group C (Antibiotic) compared with the neutrophil count of Group B (Probiotics) /x400. Results shows comparison between Group B and A were Mean \pm SD of numbers of Neutrophils / x400 in groups B1 were 7.2700 ± 6.26085 , B2 5.2950 ± 6.02407 , B3 2.7725 ± 3.37920 , B4 2.5000 ± 1.29099 and in A1 were 23.2500 ± 2.21736 , A2 29.7500 ± 4.64579 , A3 25.0000 ± 7.11805 and A4 15.0000 ± 3.55903 . The significant value was in B1 and A1 (P = 0.000), B2 and A2 (P=0.000), B3 and A3 (P=0.000) and B4 and A4 (P=0.000). The result Showed decreased number of Neutrophils in Group B in comparison with A. Results of comparison between Group B and Group C were Mean \pm SD of numbers of Neutrophils / x400 in groups B1 were 7.2700 ± 6.26085 , B2 5.2950 ± 6.02407 , B3 2.7725 ± 3.37920 , B4 2.5000 ± 1.29099 and C1 22.0000 ± 8.12404 , C2 20.0000 ± 2.44949 , C3 $18.0000 \pm .81650$ and C4 10.7500 ± 2.21736 . While comparing the group B1 and C1 p value was (P=0.001), B2 and C2 (P=0.001), B3 and C3 (P=0.001) and B4 and C4 (P=0.001).

Conclusion: Probiotics shows strong anti-inflammatory status in comparison with other therapeutic groups.

Key Words: Probiotics, Cutaneous, Healing, Comparison.

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INTRODUCTION

Skin is a part of Integumentary system and a protective barrier performs multiple functions in the body like, protection from the external atmosphere and maintains the body homeostasis, prevents from abrasions and from the influence of harmful physical and chemical substances, solar radiation and invading of microorganisms.¹ Skin is a potent external barrier, prevent the human body health through its strong immune potential.^{2,3} Wound is a damage of the cellular and molecular configuration of the body, which creates an interruption in the cellular, anatomical structures and functions.⁴

In (1992) codex Alimentarius⁴ describe yogurt as milk derivative that obtained from fermentation of lactic acid present in milk by means of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. This fermented dairy product contain various amount of microorganism related with different families, that end product is lactic acid. Probiotics are microbes that are naturally exist within the human body and support with its healthy execution.⁶ The meaning of probiotics has been redefined right through the time, and it was defined as “live organism that give a healthy effect on the host when devoted in a sufficient amount”.⁷ Most significant effects seen on dermal health for example improving the dermal healthy condition in eczema, atopic dermatitis, wound repairing and scar especially burn wounds, potentiate the restoration of skin and enhance its immunity.⁸⁻¹¹ They had an extreme role in the treatment of multiple bacterial infections.¹² Some of the studies documented about function of Lipoteichoic acid as pathogenic associated molecular base that help in secretion of pro-inflammatory cytokines and other inflammatory mediators.¹³ In some of newly studies seen that *Lactobacilli* and *Bifidobacteria* composed of maximum concentration of LTA that promote the skin protective mechanism against bacterial infection.¹⁴

In our study for the topical use of antibiotic is the Neomycin, belonging to the family of aminoglycoside chemotherapeutic group. Basically it is bactericidal agent, inhibit the protein synthesis that change the ribosomal activity and show the significant results against gram negative microorganisms.¹⁵

MATERIAL & METHODS

This study was carried out in the department of Anatomy of Al-Tibri Medical Collage Isra University Karachi campus, from June 2013 to October 2013. Total sixty numbers of Albino rats were taken from the animal house of Al-Tibri medical College and divided into three groups on the bases of topical applications. Group A topically applied Normal Saline, Group B topically applied Probiotics and Group C

topically applied antibiotic (Neomycin) once daily for 21 days. They were randomly divided into four subgroups according to day of sampling, A1, B1 and C1 (day03), A2, B2 and C2 (day 07), A3, B3 and C3 (day14) and A4, B4 and C4 (day 21). Only healthy animals of either gender were selected and diseased animals were excluded out. Wound formation; After grouping the wound were created after given deep anesthesia using ether, clean and shaved the dorsal surface of the rats about an area of $1.5 \times 1.5\text{cm}^2$ and rats were kept in an individual cage.

Strains of *Lactobacillus*: They were isolated from yogurt. The *Lactobacilli* strain were identified and authenticated from the department of Microbiology of PCSIR Karachi. *Lactobacillus* spp was isolated from yogurt, which was purchased from the market every day and was homogenized using a vortex mixer. Ten folds dilution were made using phosphate buffer saline. Serial of dilutions were spread on the plates of MRS agar and incubated at 35°C for 48 hours. Isolated colonies were streaked onto fresh agar plates. Purity of isolation was checked by microscopic examinations. Isolation was gram stained and tested for catalase production test. *Lactobacillus* catalase negative were used for study. Pure culture of *Lactobacillus* was grown in MRS broth for the studies in rat model. In order to prepare the ointment 1010 to 1011 CFU/ml bacteria that were collected every day after 48 hours of culture and used in liquid form by direct application of wound.

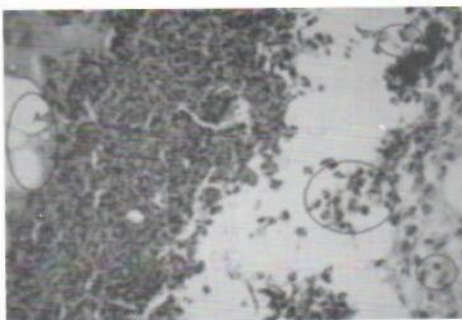
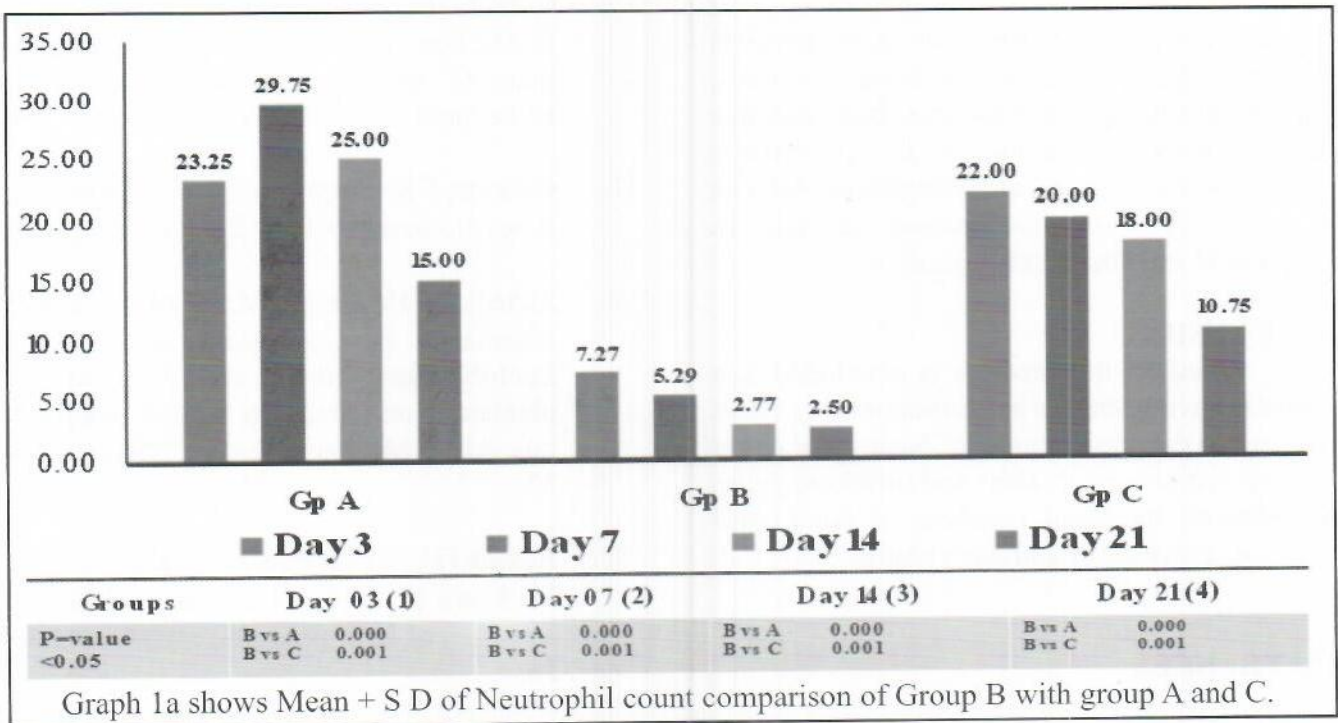
The sample were taken on day 3, 7, 14 and 21 and the tissue from the wound site including whole thickness of skin and surrounding skin was removed. These samples were fixed in 10% formaldehyde solution. Tissue were processing and staining for the microscopic examination. The study was conducted in accordance with a protocol approved by Ethical committee of Isra University.

RESULT:

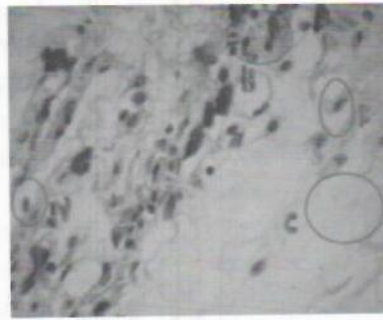
In this study we observed Neutrophil count/ $\times 400$. The results were put SPSS version 17 and data were evaluated through One Way ANOVA post hoc Tukey's test. We compare the neutrophil

count of Group A (Control) and Group C (Antibiotic) from the neutrophil count of Group B (Probiotics) /x400. Resulte shows comparison between Group B and A were Mean ± SD of numbers of Neutrophils / x400 in groups B1 were 7.2700 ± 6.26085, B2 5.2950 ± 6.02407, B3 2.7725 ± 3.37920, B4 2.5000 ± 1.29099 and in A1 were 23.2500 ± 2.21736, A2 29.7500 ± 4.64579, A3 25.0000 ± 7.11805 and A4 15.0000 ± 3.55903. The significant value was in B1 and A1 (P = 0.000), B2 and A2 (P=0.000), B3 and A3 (P=0.000) and B4 and A4 (P=0.000) came to be out as shown in graph 1a and Figure 1.1, 1.2 and 1.3. Shows decreased number of Neutrophils in

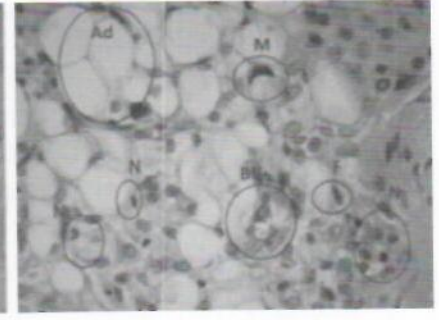
Group B in comparison with A. Results of comparison between Group B and Group C were Mean ± SD of numbers of Neutrophils / x400 in groups B1 were 7.2700 ± 6.26085, B2 5.2950 ± 6.02407, B3 2.7725 ± 3.37920, B4 2.5000 ± 1.29099 and C1 22.0000 ± 8.12404, C2 20.0000 ± 2.44949, C3 18.0000 ± .81650 and C4 10.7500 ± 2.21736. The significant value was in B1 and C1 (P = 0.001), B2 and C2 (P=0.001), B3 and C3 (P=0.001) and B4 and C4 (P=0.001) came to be out as shown in graph 1a and Figure no 1.4, 1.5 and 1.6. Shows decreased number of Neutrophils in Group B in comparison with C.



Photomicrograph 1.1 shows (H&E stain) shows increased no of neutrophil/x400. In group A.Ad (adipose tissue) N (Neutrophil) & BV (Blood vessels)



Photomicrograph 1.2 shows (H&E stain) shows increased no of neutrophil/x400. In group B.N (Neutrophil) F



Photomicrograph 1.3 shows (H&E stain) shows increased no of neutrophil/x100. In group C.N (Neutrophil) F(Fibroblast) AD and BV

DISCUSSION

The treatment of wound repaired and skin loss is one of main problem in surgical setting. The therapeutic agent which is chosen for the treatment of wounds should preferably accelerate the one or more phases of healing without minimal unto wanted effects. Lactic acid is a bioactive property of probiotics that's prevents the production of pathogenic bacteria. Reduction of Neutrophils count can measure in lactobacillus treated animals due to decrease inflammation and bacterial adhesion¹⁶. On day 03 inflammatory phase of wound repair shows increase numbers of neutrophils count, but in recovery phase of probiotics treated wound showed marked decrease numbers of neutrophils in comparison between control normal saline treated animals, antibiotic treated group of animals. In accordance with Heidari et al and Tajabady et al^{17,18}, our results showed reduce numbers of neutrophils at the site of injury in probiotics treated animals in comparison with therapeutic agents.

CONCLUSION

Based on this study it is concluded that probiotic having stronge anti-inflammatory effect evidence as decrease number of Neutrophil count as compared to normal saline and antibiotic group that showed increased numbers of Neutrophil count that showed inflammatory phase.

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