

# Probiotics Accelerate the Process of Neovascularization in Wound Healing: A Comparative Study in Rats

Probiotics  
Accelerate the  
Process of  
Wound Healing

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## ABSTRACT

**Objective:** To assess the role of probiotics in accelerating the process of Neovascularization in wound healing.

**Study Design:** Experimental Study

**Place and Duration of Study:** This study was conducted at the Al-Tibri Medical College and Hospital, during May 2018 to December 2018.

**Materials and Methods:** Total 18 male albino rats were selected through randomized sampling and divided into three groups; Group A was given topical normal saline, Group B was given topical probiotic (Lactobacillus Acidophilus), and Group C was given topical antibiotic. The interventional agent was given once for 21 days. Samples were taken on the 3<sup>rd</sup>, 7<sup>th</sup>, 14<sup>th</sup>, and 21<sup>st</sup> days to observe under the microscope at 400x. Data were analyzed using SPSS Version 20.0, and the mean between the groups was compared using ANOVA and post hoc Tukey's test. A P-value of  $\leq 0.05$  was considered statistically significant.

**Results:** Neovascularization was seen most among the group B candidates that were given topical probiotics. A significant difference (P-value  $\leq 0.05$ ) existed when comparing Group B with Group A and comparing Group B with Group C.

**Conclusion:** The results revealed the effective role of topical probiotic in accelerating the process of neovascularization, and due to their potent anti-inflammatory effects the probiotic accelerates the early availability of immune cells at site of healing and faster the process of repairing.

**Key Words:** Wound, Probiotic, Neovascularization, Healing

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## INTRODUCTION

Wound damage to the epithelial barrier in the superficial or deep layer of skin requires repairing through the generalized process of healing. There are so many factors that can accelerate or delay the healing process. The healing process comprises extracellular interactions, acceleration of chemical mediators, and availability of inflammatory cells at the site of healing<sup>1-2</sup>. Repairing of the wound can be assessed through biological, chemical, or histological parameters<sup>3-4</sup>. The healing process mainly depends on the balance between the host and the microbes.

Naturally, the body with different compositions and distributions of the host microorganisms may help in healing and maintain immunity like microbes from intestinal flora<sup>5-6</sup>. Currently, there are so many therapeutic agents available for wound repair, and surgeons or physicians can adopt different strategies to fasten the healing process, either topically and orally like topical antibiotics, anti-inflammatory, and probiotics. The probiotics are the organisms that ferment energy substrates that can be resistant to multiple pathogens. By WHO, probiotics are a live organism that can administer in an adequate amount, providing healthy and beneficial effects<sup>7-8</sup>. Commercially different compositions of prebiotics, probiotics, and symbiotics can be isolated for the various resources in the form of different species<sup>9</sup>. The most common species used by the clinicians are Lactobacillus, and can modulate the healing process<sup>10</sup>. The study's objective is to evaluate the role of topical probiotics in the acceleration of the neovascularization process of wound repair compared with topical antibiotics and steroids. Due to unhealthy environmental conditions, the requirement of early wound closure is an essential part of therapy, and prevention from late complications can be hold by taken such benefits from this research.

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## MATERIALS AND METHODS

An experimental study was designed at Al-Tibri Medical College and Hospital, Isra University. Karachi campus. A total of 18 male albino rats were selected through randomized sampling, and they were subdivided into three groups based on topical treatment. Each group comprises of 6 animals and weight between 150 to 250mg. After taking ethical approval from the concerned ethical committee, the study was conducted from May 2018 to December 2018. Group A was given topical normal saline once daily (control group), Group B was given probiotic topically containing  $10^{10}$  or  $10^{11}$  CFU/ml, and Group C was given topical antibiotic (neomycin cream) once daily for 21 days. The probiotic (*Lactobacillus Acidophilus*) were isolated from yogurt, and for that purpose, the fresh yogurt was purchased from the market, and organisms were isolated by the department of Microbiology (PCSIR) Karachi. The organisms were grown on MRS agar plates and incubated at  $34^{\circ}\text{C}$  for the next 48 hours. For the confirmation of the bacterial species, a catalase test was performed because the lactobacilli are catalase-negative. The fresh culture was taken after every 48 hours, and liquid form was given as a topical application in Group B. Wound was formed at the dorsal surface of the rat with the area of  $2 \times 2 \text{cm}^2$ , after given anesthesia with ether and then the animals were kept in separate cages with tagging of groups. During the study, the different samples were taken on various days, like day 3, 7, 14, and 21. The sample of the dermis was taken from the site of the wound to observe the healing process. The microscopic slides were prepared to assess the skin's microscopic features through a light microscope at 400x. In this study, we observed the numbers of blood vessels as a process of Neovascularization among different therapeutic groups. The data were analyzed through SPSS version 20.0. To compare the mean between the groups, the ANOVA was applied along with post hoc Tukey's test. The level of significance was considered  $P < 0.05$ .

## RESULTS

Figure: 1.1 shows the Mean numbers of blood vessels among different therapeutic groups on Day 3, 7, 14, and 21.

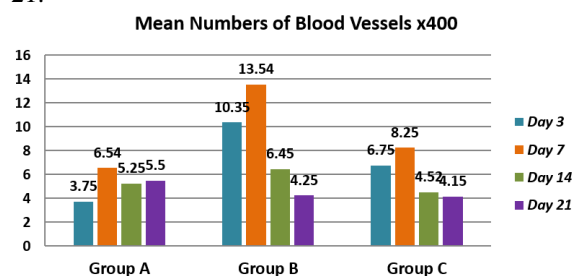


Figure No.1: Shows the Mean numbers of blood vessels among different therapeutic groups on Day 3, 7, 14 and 21

Table No. 1: Shows level of significance between the different therapeutic groups

| Groups | Day 3 | Day 7 | Day 14 | Day 21 |
|--------|-------|-------|--------|--------|
| B vs A | 0.001 | 0.001 | 0.001  | 0.001  |
| B vs C | 0.001 | 0.001 | 0.001  | 0.001  |

One Way ANOVA Post hoc Tukey's test applied

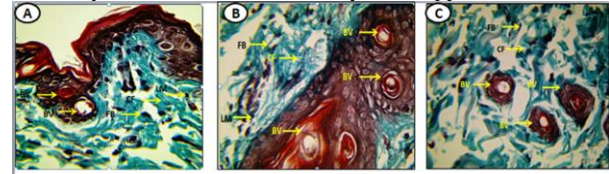


Figure: 1.2 shows Photomicrograph of Histological slides (Trichrome stain) x400 of Skin tissue on Day 7. BV: Blood Vessels, FB: Fibroblast, CF: Irregular Collagen Fibers and LM: Lymphocytes

Figure No.2: Photomicrographs of histological slides of skin x400 on day 7 among the different groups.

Table 1. Shows the level of significance between the different therapeutic groups.

Figure: 1.2 shows photomicrographs of histological slides of skin x400 on day 7 among the different groups.

## DISCUSSION

Microbial colonization can occur immediately after the development of a wound. The bacterial load can have a significant impact on wound healing. Potential new talk about therapeutic agents is ongoing, regulating the microbes, pathogens, and the body's immune system, thereby accelerating wound healing<sup>11</sup>. Lactobacillus treat wounds resulted in reduction of wound size at favorable time period when compared with the group treated with normal saline. Our study successfully showed that probiotics resulted in efficient contraction of wound area when compared with the other groups in our study. The results showed that Lactobacillus successfully helped in speeding up angiogenesis and repair. One of the researchers used different species of Lactobacilli (*Lactobacillus Plantarum* and *Lactobacillus Brevis*) but showed similar results to our study in which the interventional groups experienced reduced in the number of neutrophils and acceleration of wound healing<sup>12</sup>.

Productions of white blood cells such as macrophages were stimulated by Lactobacillus. New fibroblast cells proliferation and abundance were also increased. These two cells synergistically helped in neovascularization. New blood vessel formation contributes in the healing process of the wound along with the formation of granulation tissue in a process called hemostasis. Hemostasis is necessary for proper healing and provides access to inflammatory cells at the repair site. The probiotics treated groups showed the best hemostasis action amongst all the groups. Lactobacilli have shown to suppress the inflammation in animal models of experimental colitis, and this can also be seen

in our study as the inflammatory phase of wound healing subsides more quickly. The proliferative phase, which results in granulation tissue formation and angiogenesis, took place much earlier, thereby accelerating wound healing<sup>13-14</sup>.

Per Tamawski et al. 2005, probiotics may even help in stimulating growth factors and cytokines. Due to this, increased migration of fibroblast cells can occur at the site of injury. Lactobacillus treated wounds had a higher quantity of fibroblast cells compare to the rest of the groups. Fibroblast is important and helps in the synthesis of collage as well as areolar tissues. Fibroblast and macrophages help in angiogenesis at the site of damage. Fibroblasts favor the formation of new collagen fibers, as well as new vessel formation. These factors are critical in helping earlier re-epithelialization of tissue.<sup>15</sup> This re-epithelialization process was also shown by Mehrabani et al.: 2010, who used Rhus Coriaria extracts and showed an acceleration in wound healing with its effects due to an increase in re-epithelialization, collagen deposition and a decrease in MPO activity<sup>16</sup>. This treatment can also be beneficial in individuals who suffer from systemic complications from wound healing such as diabetes, as shown in the study by Mohseni et al. 2017<sup>17</sup>. Furthermore, Campos et al. 2020 also showed in diabetic rats that perioperative supplementation with probiotics resulted in better skin wound healing<sup>18</sup>. This can once again be related to a reduction in the inflammatory phase of wound healing and increased Neovascularization and type I collagen deposition, this is in accordance with our study. This strongly suggests that probiotics can be used in diabetic patients with impaired wound healing due to persistent inflammatory cytokines being developed. Probiotics should strongly be considered as a strong contender as a therapeutic agent in wound healing. Our previous studies have also shown that its Neovascularization, anti-inflammatory ability, and wound healing acceleration make it a robust therapeutic agent for people with wounds<sup>19</sup>. However, it must be noted that although it is a very viable agent in wound healing, there are other treatment options available such as Low-level laser therapy, which has been proven to be more effective than probiotics<sup>20-21</sup>. More studies are required to assess probiotics with other agents used in wound healing and test them in individuals with local and systemic complications that may delay wound healing and see what effect probiotics may have in these parameters.

## CONCLUSION

The results revealed the effective role of topical probiotic in accelerating the process of neovascularization, and due to their potent anti-inflammatory effects the probiotic accelerate the early availability of immune cells at site of healing and faster the process of repairing.

### Author's Contribution:

|                            |  |
|----------------------------|--|
| Concept & Design of Study: | Hina Khan  |
| Drafting:                  | Shagufta Memon, Shahid Korai                       |
| Data Analysis:             | Ahmed Mujtaba Memon, Farheen Hameed, Shahid Kamran |
| Revisiting Critically:     | Hina Khan, Shagufta Memon                          |
| Final Approval of version: | Hina Khan  |

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

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