AIJPS
Academic International Publishers
Academic International Journal of Pure Science (E. ISSN: 2984-7737)
Aca. Intl. J. P. Sci. 2025; 03(1):47-57.

Comparative Histological Changes to Investigate the Therapeutic Effect of Cicalfate+ cream and Penicillin-Streptomycin on Wound Healing in Rabbits

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(Received 10 January 2025, Revised 4 February 2025, Accepted 28 March 2025, Published 21 May 2025)

Abstract

The present study was designed to evaluate the comparative effect of Cicalfate+ cream and penicillin-streptomycin on the histology of rabbits' healing processes in full-thickness skin wounds. All rabbits were forty-five healthy adult bucks weighing between 1.3 and 1.8 kg were made A full-thickness wound measuring 2.5 x 2.5 cm was made in the dorsal back area. Intramuscular injections of xylazine hydrochloride (5 mg/kg), ketamine hydrochloride (35 mg/kg) were given very quickly (IM), along with 1 mg/kg of diazepam. These animals were separated into three groups (A, B and C groups) The first group was treated intramuscular with penicillin-streptomycin daily with single dose (group A), and the second group was given cicalfate+ cream topically twice daily (group B), the last group designated as (group C) served as the control and didn't receive any treatment. For histopathological evaluation, on the 3rd, 7th, and 14th days after wounding. Histological analysis demonstrated that wound healing was more organized and efficient in group (B) compared to the other groups, with group (A) showing a moderate degree of improvement, and the rate of healing highly in group (B) than other groups which led to highly attachment between layers of skin resulting in good blood supply and nutrition also greater cellularity and finally faster healing to the wound.

Keywords:
Wound healing, Postbiotic Cicalfate+ cream, penicillin-streptomycin, rabbits.

How to cite:
Hayder Nadhim Alkhalissi. Comparative histological changes to investigate the therapeutic effect of cicalfate+ cream and penicillin-streptomycin on wound healing in rabbits. *Aca. Intl. J. P. Sci. 2025;03(1):47-57.* https://doi.org/10.59675/P316

Introduction

Skin consists of two main layers: the superficial epidermis and the dermis underneath. The epidermis exhibits five layers, which are stratum basale, stratum spinosum, stratum granulosum, stratum lucidum, and stratum corneum, while the dermis contains cellular components of epidermis origin and connective tissue fibers [1]. Wound conditions are prevalent in various countries and are often associated with microbial complications, such as localized or systemic infections, dissemination of multidrug-resistant organisms, and impaired wound healing [2]. A wound is a disruption of the normal contiguity or continuity of body structures caused by a physical wound [3].

Skin wounds refer to injuries that disrupt the structural integrity and physiological function of the skin. They are typically classified as either acute or chronic based on the duration and progression of the healing process. Acute wounds may vary in severity, from superficial abrasions to deep tissue injuries, and generally follow a normal repair mechanism, with complete healing commonly occurring within three weeks [4, 5, 6]. The wound healing in skin is an all-around coordinated endurance instrument that can be impacted by different conditions, leading to a superior or worse course of healing [7].

Classical experimental use of rabbits includes improvement of new surgical techniques, antibody production, toxicity, and physiology studies for the testing of new drugs [8]. Various modalities have been suggested for wound management, including hyperbaric oxygen therapy, wound dressings, and antibiotics. Antibiotics are commonly utilized to decrease the bacterial load surrounding the wound area and are often used in conjunction with dressings to promote more effective inflammatory and proliferative phases of healing [9]. The effect of various antibiotic drugs on wound-healing was examined using the following parenteral administration in rats. The criterion for healing was the tensile strength of an incised and sutured healing skin wound [10]. Chronic wound healing represents a critical worldwide public health issue. Abuse and drug resistance of antibiotics are the key problems in the treatment of chronic wounds at this time, so the postbiotics are a novel promising strategy. Previous studies have reported that postbiotics have a wide range of activities, including anti-inflammatory abilities, antimicrobial, immunomodulatory, and antioxidant. However, several aspects related to these postbiotic activities remain poorly known or unexplored [11]. The application of probiotics and postbiotics has been shown to have a good impact on the conservation of skin health by inhibiting the growth of harmful bacteria and allowing the growth of beneficial bacteria. Probiotics work by adhering to the skin and mucosal membranes and engaging in competition against pathogenic organisms for nutrients, which prevents the growth of pathogenic bacteria, resulting in improved skin health [12, 13, 14]. According to the histological findings, the postbiotic film accelerated wound healing by modulating the inflammatory phase, promoting collagen and elastin deposition, and enhancing angiogenesis [15]. In search of natural products capable of regulating the immune response are postbiotics, which are soluble compounds secreted by probiotics for individuals that have not been studied or tested [16, 17].

A cicalfate+ cream which is a repairing protective cream has active ingredients like zinc sulfate and copper sulfate act as a physical anti-bacterial and zinc oxide which is a protective agent also Avene thermal spring water with soothing, anti-irritant and anti-inflammatory properties, on other hand *Aquaphilus dolomiae* which is a unique bacteria present in Avene thermal spring water with a biotechnological extraction of this organism resulting in a new postbiotic called (C+ Restore postbiotic), finally this cream for its formation makes skin recovery four times faster [18].

Materials and Methods

Animal models

A total of forty-five adult male rabbits, weighing between 1.3 and 1.8 kg, were utilized in the present study. The animals were randomly allocated into three groups, with fifteen rabbits in each group. All animals were housed in individual cages at the animal facility of the College of Veterinary Medicine, University of Kerbala, and maintained under standardized conditions of environment, nutrition, and management throughout the experimental period. All rabbits were completely examined clinically every two days during the experimental period to ensure they were healthy and in good condition.

Operative procedure

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Animals were fasted for 6–12 hours before surgery, with water withheld for 5 hours. The dorsal back region was shaved and aseptically prepared. General anesthesia was induced following premedication with diazepam at a dose of 1 mg/kg body weight. Ten minutes later, xylazine hydrochloride (5 mg/kg BW) and ketamine hydrochloride (35 mg/kg BW) were administered intramuscularly to achieve surgical anesthesia [19].
The animals were placed in a ventral recumbent position, exposing aseptically the dorsal surface of their back for the surgery. A complete thickness cutaneous wounds measuring 2.5 × 2.5 cm was surgically induced in the mid-dorsal thoracic region of each animal (fig.1). The experimental animals were assigned to three groups, The first group was given of penicillin-streptomycin injection a single intramuscular dose of procaine penicillin G (80,000 U/kg of body weight) plus probenecid (25 mg/kg of body weight), together with streptomycin (20 mg/kg of body weight) daily for 5 days (group A) [20], and the second group was given of cicalfate+ cream topically twice daily for 14 days (group B), while the last group the animals were remained wounds untreated serving as a reference or standard control group (group C).

In the present study, histological assessment was conducted on days (3, 7, and 14) following treatment in the experimental groups, with the same time points applied to the controlled group. A specimen of skin (2.5×2.5) cm full-thickness were obtained and fixed and immersed in 10% formalin solution and subjected to routine tissue processing techniques and stained with normal stain which hematoxylin-eosin stain (H&E) [21].

The cicalfate+ cream which is a repair cream composed mainly from the following main active ingredients:- (C+ Restore 1% postbiotic, copper sulfate 0.2%, zinc sulfate 0.1% zinc oxide 4% and avene thermal water 45%), this cream is a postbiotic cream with a therapeutic properties of repairing, antibacterial, soothing, anti-irritant and anti-inflammation effects.

Results

The results of current study showed changes in the histological statement of wounds seemed actually that the thickness of stratum corneum of group (A) increased slight gradually starting from third day until seventh day which was a significant difference ($P \leq 0.05$) were (7.11±0.15 & 9.17±0.15 µm) respectively (fig.2 and 3) but this increase decreased noticeably on fourteenth day and became (4.35±0.25 µm) (fig.4), the slight increasing of this layer to facilitate migration of keratinocytes and allows cells to spread and close the wound but the decrease in thickness of stratum corneum at the end of this study made wound repair less than ideal because we need thick stratum corneum to prevent fluid loss and maintain moisture with a protection against microbes thus preserving the wound (table 1), while the thickness of stratum corneum of group (B) also increased slight gradually from the third day to the fourteenth day with a significant difference ($P \leq 0.05$) were (5.25±0.33 & 7.18±0.26 & 16.22±0.18 µm) respectively (fig.5,6 and 7), the slight and unnoticeable increasing of stratum corneum also promote migration of keratinocytes and allows cells to spread faster to close the wound and the noticeable increasing at this layer in the fourteenth day helped in maintain moisture and prevent fluid loss and a good protective layer against microbes thus the wound healed better due to its high moisturizing properties from its components like Avene thermal spring water also the copper sulfate and zinc sulfate gave a protective layer on wound which act as a physical anti-bacterial and allow wound in contact with atmosphere so it gets oxygen normally without gets infected also due to the action of zinc oxide as a protective agent, and the repairing activity to the wound which came from (C+ Restore postbiotic) that comes driven with a biotechnological extraction of *Aquaphilus dolomiae* that found in Avene thermal spring water in cicalfate+ cream

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(table 1). On the other hand the thickness of stratum corneum of group (C) increased progressively from third day until it reaches its maximum thickness on the fourteenth day with a significant difference ($P \leq 0.05$) were (22.25±0.49 & 40.85±0.76 & 48.03±0.14 µm) respectively (fig.8,9 and 10) this believed lead to difficult in migration of keratinocyte to the surface and also in spread of cells this causes delayed wound healing with scar tissue formation more than other groups (table 1).

The histological inspection revealed that there was increasing of stratum spinosum of group (A) from third day gradually until seventh day with a significant difference ($P \leq 0.05$) were (6.28±0.31 & 17.35±0.43 µm) respectively (fig.2 and 3), then decreased till the end of study was (4.34±0.38 µm) (fig.4), that mean the number of cells which migrated to the upper layers was few (table 2), while the thickness of stratum spinosum of group (B) was increased progressively especially at fourteenth day were (10.04±0.13 & 18.25±0.36 & 57.37±2.52 µm) respectively (fig.5,6 and 7), that mean there was excessive increase in number of cells which migrated toward upper layers and that helps in wound repair and healing better (table 2). On the other hand the increase of stratum spinosum of group (C) was little from third day until the end of this study with a significant difference ($P \leq 0.05$) were (4.18±0.30 & 5.16±0.26 & 6.26±0.27 µm) respectively (fig.8,9 and 10) this led to slowly healing of wound (table 2).

The histological picture showed that there was a significantly increase ($P \leq 0.05$) in dermis of group (A) from third day until seventh day were (117.24±0.31 & 241.27±1.20 µm) respectively (fig.2 and 3) then decreased until fourteenth day which was (85.32±0.38 µm) (fig.4) that showed the provide of supplements to the epidermis was not perfect and that affected on wound healing (table 3). while there was a progressive increasing in thickness of dermis of group (B) especially at the end of study with a significant difference were (160.18±0.81 & 170.30±0.49 & 188.35±0.49 µm) respectively (fig.5,6 and 7) which was the most increasing between the other groups and that revealed there was more blood supply and other supplements needed for wound healing (table 3). On the other hand there was a marked significant increase ($P \leq 0.05$) of dermis of group (C) which was gradually from third day until seventh day were (77.60±0.76 & 80.14±0.72 µm) respectively (fig.8 and 9) then reduce the thickness of dermis until end of this study was (44.18±0.17 µm) (fig.10), that mean there was insufficient supplements which needed for epidermis for wound repair and this led to delayed in wound healing (table 3).

It can be argued that this study showed the dermal papillae were markedly high in group (B) more than other groups (fig.5,6 and 7), that means increasing area of contact between epidermal and dermal layers which enhanced the resistance of mechanical sharing and promotes dermal-epidermal adhesion resulting in increased structural and mechanical constancy of the epidermis and this promoted the transfer of nutrients from the dermal microvasculature to the epidermal layer.

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Fig.1. Gross area of full-thickness wound was created in the dorsal back region.

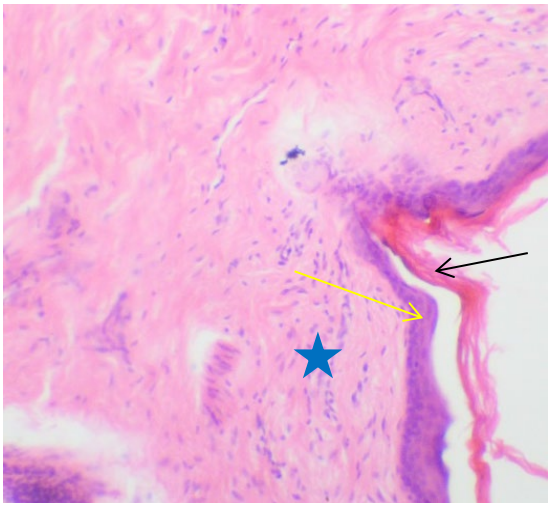

Fig.2 Microscopic cross view of group (A) at day 3 showed stratum corneum (black arrow), stratum spinosum (yellow arrow), and dermis (blue star). (H&E X100).

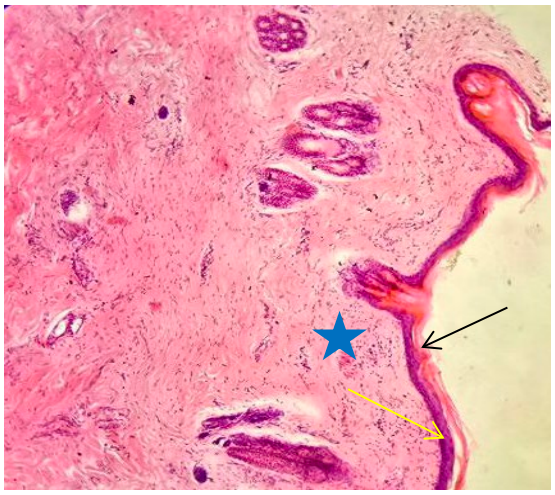

Fig.3 Microscopic cross-section of group (A) at day 7 showed stratum corneum (black arrow), stratum spinosum (yellow arrow), and dermis (blue star). (H&E X100).

Fig.4 Histological cross-section of group (A) at day 14 showed stratum corneum (black arrow), stratum spinosum (yellow arrow), and dermis (blue star). (H&E X100).

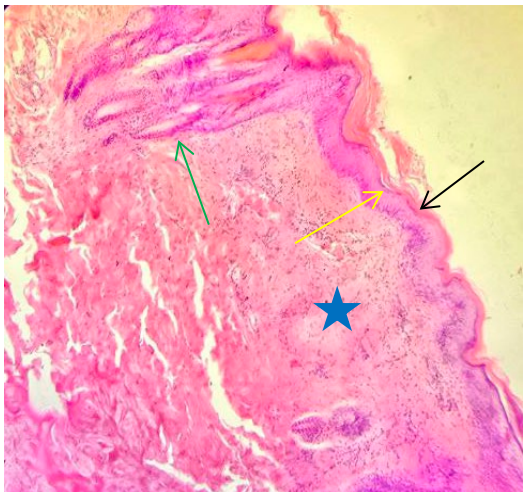

Fig.5 Histological cross-section of group (B) at day 3 showed stratum corneum (black arrow), stratum spinosum (yellow arrow), dermis (blue star), and dermal papillae (green arrow). (H&E X100).

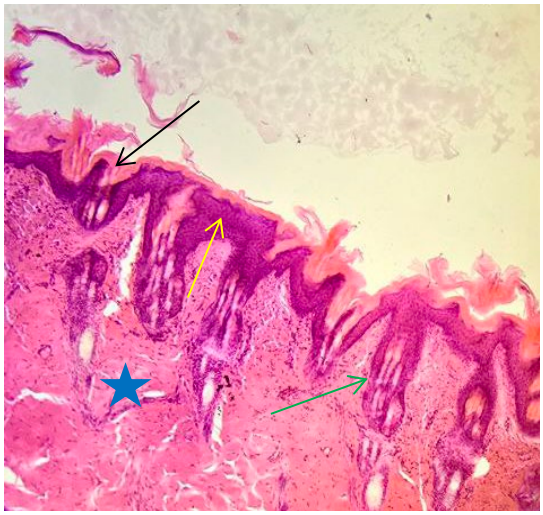

Fig.6 Histological cross-section of group (B) at day 7 showed stratum corneum (black arrow), stratum spinosum (yellow arrow), dermis (blue star), and dermal papillae (green arrow). (H&E X100).

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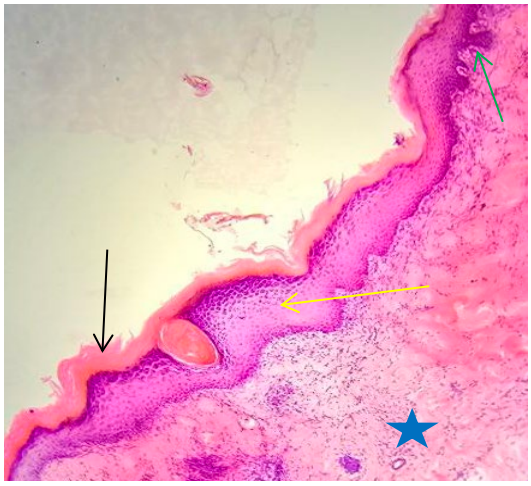

Fig.7 Histological cross-section of group (B) at day 14 showed stratum corneum (black arrow), stratum spinosum (yellow arrow), dermis (blue star), and dermal papillae (green arrow). (H&E X100).

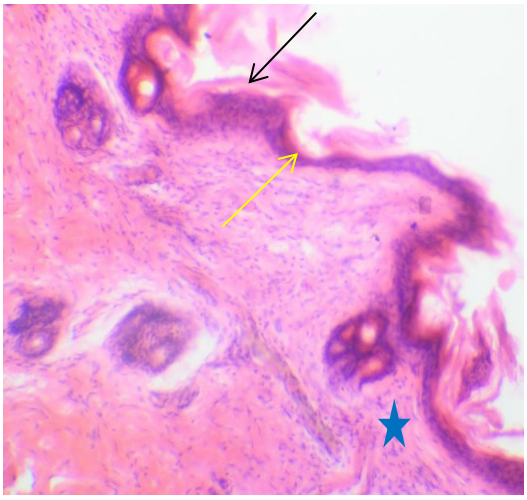

Fig.8 Histological cross-section of group (C) at day 3 showed stratum corneum (black arrow), stratum spinosum (yellow arrow), and dermis (blue star). (H&E X100).

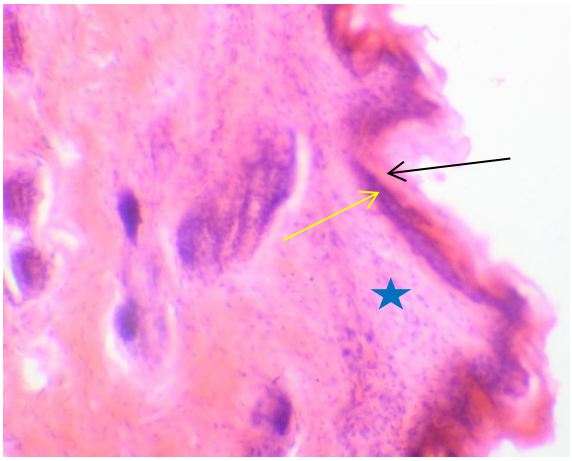

Fig.9 Histological cross-section of group (C) at day 7 showed stratum corneum (black arrow), stratum spinosum (yellow arrow), and dermis (blue star). (H&E X100).

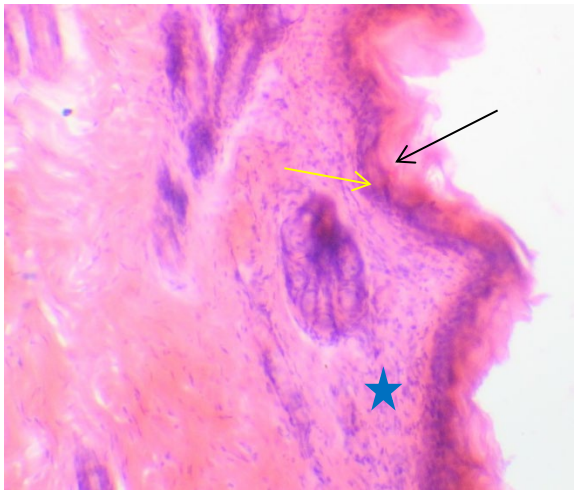

Fig.10 Histological cross-section of group (C) at day 14 showed stratum corneum (black arrow), stratum spinosum (yellow arrow), and dermis (blue star). (H&E X100).

Table 1: Thickness of stratum corneum between groups.

Groups	Day 3 (Mean ± SD) (unit µm)	Day 7 (Mean ± SD) (unit µm)	Day 14 (Mean ± SD) (unit µm)
Penicillin-streptomycin (Group A)	7.11 ± 0.15	9.17 ± 0.15	4.35 ± 0.25
Cicalfate+ (Group B)	5.25 ± 0.33	7.18 ± 0.26	16.22 ± 0.18
Control (Group C)	22.25 ± 0.49	40.85 ± 0.76	48.03 ± 0.14

Table (2) The thickness of stratum spinosum between groups.

Groups	Day 3 (Mean ± SD) (unit µm)	Day 7 (Mean ± SD) (unit µm)	Day 14 (Mean ± SD) (unit µm)
Penicillin-streptomycin (Group A)	6.28 ± 0.31	17.35 ± 0.43	4.34 ± 0.38
Cicalfate+ (Group B)	10.04 ± 0.13	18.25 ± 0.36	57.37 ± 2.52
Control (Group C)	4.18 ± 0.30	5.16 ± 0.26	6.26 ± 0.27

Table 3: The thickness of dermis between groups.

Groups	Day 3 (Mean ± SD) (unit µm)	Day 7 (Mean ± SD) (unit µm)	Day 14 (Mean ± SD) (unit µm)
Penicillin-streptomycin (Group A)	117.24 ± 0.31	241.27 ± 1.20	85.32 ± 0.38
Cicalfate+ (Group B)	160.18 ± 0.81	170.30 ± 0.49	188.35 ± 0.49
Control (Group C)	77.60 ± 0.76	80.14 ± 0.72	44.18 ± 0.17

Discussion

In the current study, the histological observations of wound initiation of the healing process were observed in all groups; however, the progression was more advanced in group (B). The stratum corneum gradually noticeable increased in group (B) and this beneficial for the wound healing, this agreed with [22] who saw that the alterations in epidermal differentiation and lipid profile of

keratinocytes compromise the integrity of the skin barrier, facilitating the penetration of environmental allergens and triggering immune responses and skin inflammation.

The present investigation was to evaluate and compare the changes of antibiotics and postbiotics on the healing of complete-thickness skin wounds in the rabbits and better epithelization in comparison with the controlled group. Histological examination of the wounds in current study indicated that the degree of progression of stratum spinosum was increased in all treated and controlled groups, while the development was more in treated groups especially in postbiotic group (group B) and in less degree in controlled group (C) and that thought due to pharmacological effects of cicalfate+ cream which contains C+ restore postbiotic, zinc sulfate and Avene thermal spring water, this similar observations to [23] who observed that the zinc is a cofactor for many metalloenzymes required for cell membrane repair, cell proliferation and growth and thus for successfully wound repair, and agreed with [24] who shown that the importance of zinc concentrations towards healing wounds in patients. Also [25] suggested that topical zinc therapy was importance in autodebridement, anti-infective action, and promotion of epithelialization, and a similar finding was witnessed by [26] who used apigenin cream which is a postbiotic cream was significantly improved wound healing after the seventh day in comparison to other groups in study due to its properties on enhancing the re-epithelization process and collagen fibers deposition in dermis, also agreed with the result recorded by [27] who observed that was statistically significant wound healing differences used thermal water which had an effective role in wound healing, while disagreed with [28] who noticed that there were no statistically significant differences in growth or serological or other response except on immune response which affected and modulate little in wild rabbits treated with oral postbiotics. The increase in stratum spinosum of the wounds of the treated groups more to the control group was useful for rapid wound healing, and this agreed with [29], who noticed that epithelization, keratinization, granulation, and angiogenesis were more in the treated group compared with controlled group, and this is necessary for better wound healing.

The present study revealed that the marked increase of dermis of treated groups, especially in group (B) in compare with group (C), was necessary for improved wound healing and agreed with [30], who recorded that the cells of dermis, especially fibroblasts, were critical in supporting normal wound healing.

On other hand the current study showed the dermal papillae more clear and distinct in group (B) other than the rest of other groups and this agreed with [1] who explained that the dermal papillae will enhanced the resistance between dermis and epidermis through increase the surface area between them and that help skin progression.

Conclusions

Ciclofate+ cream can be used for enhancement and acceleration of skin wounds healing more than penicillin-streptomycin in rabbits. The treated groups exhibited a greater efficacy of healing than the control group.

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