



Enhancing Cutaneous Wound Healing: the Therapeutic Potential of Topical Curcumin Extract in A Rat Model



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Abstract

THIS study conducted on twenty-four Wistar rats suggests that curcumin holds promise for enhancing wound healing. Through dividing the rats into control and treated groups, along with the creation of experimental excisional skin wounds on their backs, the effects of curcumin ointment were evaluated and compared to the non-treated group. The results indicated significant improvements in wound healing among the group treated with curcumin ointment compared to the control group. Specifically, the treatment group exhibited excessive angiogenesis, which refers to the outgrowth of newly formed blood vessels, the fundamental element for providing oxygen and nutrients to the newly growing tissues. The increase in the intensity of granulation tissue could indicate improved wound-bed preparation and tissue regeneration. The enhanced epidermal re-epithelialization observed in the treated group suggests expedited wound closure and tissue regeneration. These findings underscore the multifaceted effects of curcumin, including its antioxidant, antimicrobial, and anti-inflammatory properties, which likely contribute to its positive effects on wound healing. By reducing inflammation, combating oxidative-stress, and preventing microbial colonization, curcumin creates an optimal environment for efficient wound healing. Overall, the study supports the potent effects of curcumin as a medical support in wound care, highlighting its versatility as a natural treatment option for promoting wound healing and preventing complications. However, further studies and clinical investigation are recommended to and clinical trials are necessary to advocate these findings, and to optimize the accurate dosages of curcumin for wound management in human patients.

Keywords: Curcumin ointment, topical application, wound healing, *Wistar* rat model.

Introduction

Healing of the wound is a multi-phased process that empowers the injured tissues to counteract the injurious agents and later renovate themselves. Wounds are often caused by different agents, such as mechanical, chemical, thermic, microbial, and/or antibody-antigen reaction of the dermal tissues, that

disrupt the cellular stability of the tissue. Understanding the process of wound healing and improvement is still unclear [1]. Absolutely, wound healing is indeed a multi-phased process, typically involving four main stages: including early inflammation, destruction, proliferation, and final maturation [2]. A wide variety of blood cells, such as growth factors and cytokines, play an important role

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in the restoration of damaged tissue during the wound healing process [3]. Various factors can interfere with these phases of healing, and leading to inadequate tissue restoration. Researchers suggested that factors that contribute to the degree and rate of wound healing include oxygen supply, wound-infection, ages, sexes, hormones, systemic diseases such as diabetes, using medications, alcohol, cigarette smoking and nutritional deficits [1]. A deeper knowledge of how these factors affect wound healing and the resolution of damaged wounds may lead to the development of treatment strategies that enhance wound healing and resolve delayed wound healing [4]. Curcumin is a polyphenol compound (Diferuloylmethane, 1,7-bis (4-Hydroxy-3-Methoxyphenyl)-1,6-Heptadiene-3,5-Dione) that is present within the roots of the Turmeric-plant (*Curcuma longa*). It is the main bioactive substance of the spice and, up to date, is universally used as a natural biological product in making curry powder and natural food colouring agents. All over the world, and particularly in India and China, curcumin is traditionally consumed in a daily diet, and the most current clinical trials have shown its benefits in different health fields such as cancer [5], owing to its anti-inflammatory, antioxidant, anti-proliferative, and wound-healing effects [6].

Curcumin, is a polyphenol substance, has poor-water solubility. It dissolves well in lipid-based solvents such as oil and ethanol due to its hydrophobic nature. This property presents challenges in formulating curcumin for certain applications, such as the aqueous-based pharmaceuticals or food products industries [7]. Treatment with curcumin derived substances has been found to hasten wound restoration by raising collagen, fibroblast, and vascular density, as well as acting as a pro-angiogenic activity in wound healing through activating Transforming-growth-Factor-beta (TGF- β) [8]. Different studies have shown that intra-peritoneal, oral-intake, and local topical application have significant impact on wound regeneration capacity in different areas within the body [9]. It is usually mixed with Vaseline (petroleum jelly), which acts as a vehicle for the turmeric, as well as with glycerine, which help to retain moisture by reducing trans-epidermal water loss, seeding up healing by keeping the area moist, and preventing frictional damage [10]. Therefore, the objective of this study was to investigate the influence of topical local application of curcumin on the rate of healing of the excisional sterile skin wound.

Material and Methods

Animals

Twenty-four adult male Wistar rats (*Rattus norvegicus*), whose ages ranged from 6-8 weeks were used in this experimental study. The rats were

received from the Veterinary Teaching-Hospital belonging to the College of Veterinary Medicine, University of Sulaimani, Kurdistan-region, Iraq. They are kept in standard cages and conserved hygienically according to the principle of ethical approve. They had free access to drinking water and standard food rations. This study was completed in line with the criteria and recommendations of the ethical committee of the College of Veterinary Medicine, University of Sulaimani (No. 01634).

Anesthesia and animal preparation

All surgeries were executed under the action of general anesthesia, using a sedative xylazine and anesthetic ketamine injected intra-peritoneally at doses of 5 mg/kg and 50 mg/kg, respectively. The proposed area on the back was prepared (clipped and shaved) and disinfected with povidone-iodine. An excisional circular wound on the back of each rat was created aseptically. The animals were randomly allocated into two groups, including treatment (Cur) (n = 12) and control groups (C) (n = 12).

Preparation of curcumin mixture

A formulation of 2g of turmeric powder, 2g of Vaseline and 2g of glycerin was prepared and mixed thoroughly using a spatula in a sterilized container for the treatment group. A mixture of Vaseline and glycerin were prepared as a vehicle for the control group. Each wound of each animal was applied topically with its treatment based on the group allocation every 48 hours from the first day (d0) of the excisional wound creation to the end of the experiment (d28).

Morphological Evaluation

Digital photographs of the wounds were taken following the operation at 0, 7, 14 and 28 days. The time of wound closure was estimated by defining it as a duration in which the wound became completely stuffed with new regenerated tissues and superficially re-epithelialized. Based on the photograph within the ImageJ program, the diameters of the wounds were measured at 0, 7, 14 and 28 days in the control (C) and the treatment (Cur) groups.

Histopathological Evaluation

Skin samples of approximately 2 x 3 mm at the edge of the healed excision were harvested in both groups on days 7, 14 and 28 and fixed in 10% formalin solution (Sigma Aldrich, Germany). Then four-micron-thick slice sections were prepared routinely, and processed for a microscopic slide, and then stained with H&E. The stained histological slides were analysed through an assessment of all the parameters of skin wound healing, including the intensity of inflammation, growing of granulation tissue, angiogenesis, and latent connective tissue scar formation.

Statistical analysis

The collected data were analysed using a graph pad prism (version 9.0). The means of all groups +/- SEM (Standard Error of Means) were compared using a tow-way ANOVA. The P-values of < 0.05 were considered a significant difference.

Results

The application of curcumin topically is impacting the speed at which wounds contract. The healing process of these wounds involves progressing through the primary stages of healing (Fig. 1A), at which the sizes of the excisional wounds were begin to decrease after the third-day of wound creation in the Cur and the C groups. From the third to seventh days of the topical application of curcumin, no detectable differences in wound healing were observed between the Cur and C groups. Although an obvious improvement in skin healing after 12 days was noticed in the Cur group ($P \leq 0.05$). Moreover, a complete closure of the skin defect was found in the Cur group on the 14th day, while in the C group, a small point of scar tissue was found. Finally, on the 28th day, the wounds were completely healed and disappeared in the Cur group in contrast to the C group, where small scar tissues were still left (Fig. 1B).

In addition to the macroscopical assessment of the skin wounds, the percentage of healing was estimated at the different time periods (days 0, 3, 7, 12, 14 and 28) post excisional wound creation using Image J software. Intestinally, it was recorded that the size of the wounds was decreasing in both groups. At the 3rd and 7th days post operational wound creation, there were no significant ≤ 0.05 differences in both groups. While, significant $P \leq 0.05$ differences were found at the 12th, 14th and 28th days from the application of curcumin. In the curcumin formula treated group, the degree of constriction at the 12th, 14th and 28th days with their standard deviations were (40.56 % \pm 2.27), (60.56% \pm 2.78) and (100% \pm 0) respectively, when compared to the control groups at the 12th, 14th and 28th days were (26.57 % \pm 1.19), (30.82% \pm 3.54) and (73.18% \pm 2.56) respectively using Mann-Whitney-Unpaired T-test ($p < 0.05$), (Fig. 1C).

Histopathological effects of topical curcumin administration on cutaneous wounds

Further to the macroscopical evaluation of the cutaneous wound healing, in the C-group, the wound area was assessed microscopically. On day 7, the wounds were partially closed and covered with scab (composed of clotted blood, fibrin, and polymorphonuclear cell infiltration (PMN), necrotic tissue debris). Although a marked layer of vascular granulation tissue that composed of large number of mononuclear inflammatory cell (macrophage, plasma cells, lymphocytes and fibroblasts) were found with

high numbers of neovascularization through the entire wound area, the formation of the granulation tissue was greater in the epidermal layer when compared to the dermal layer. While, in the Cur group, the wound area was completely closed and only a few scabs' necrotic tissue was seen on the wound area with fibro-vascular granulation tissue. Fig. 2 revealed arrangement of the collagen fibres randomly in different directions (disorganized), with a small number of newly formed blood vessels (angiogenesis), and an extreme inflammatory reaction such as; the presence of inflammatory cells including, neutrophils (PMN), macrophages, lymphocytes, plasma-cells, and fibroblasts in contrast to the C-group.

At day 14th, there was more progressing in wound healing (Proliferative-Phase) were seen in both groups, the wound area in the C-group showed early proliferation phase by forming a bridge of migrated cells (thin layer of keratinocytes) with fibro-vascular granulation tissue reaction that composed of large amount of thin-individual disorganized collagen fibers with few numbers of newly produced vessels and marked penetration of chronic inflammatory cells were seen in comparison to the Cur-group which revealed progress stages of proliferative phase and good quality of tissue regeneration (Fig. 3), the presence of immature hyper-plastic and non-organized epidermis that covering the wound space with moderate reactions of inflammatory cells in dermis and the hypodermis, that led to the increased of thickening of dermal layer through deposition of regularly arranged collagen fibers with fibroblast proliferation and collagen fibers were thicker, and more dense.

On day 28th, cutaneous wound sections in the control group exhibited completely uneven epidermis layer with a thin keratin layer, persistence of chronic inflammatory cells with a primary fibrous scar, and scant cells in dermal layer, as seen, vs. treated groups that presented a full organised epidermis and a thick layer of keratin. Also, sparsely distributed blood vessels were observed in the dermis with organized collagenous connective tissue (Fig. 4). Both groups showed no impact on the formation of cutaneous adnexa.

Discussion

The investigation into the wound healing potential of curcumin, when combined with Vaseline and glycerin, represents a critical endeavour in understanding its efficacy in promoting tissue repair. Curcumin's well-documented anti-inflammatory, anti-infectious, and antioxidant properties suggest its capability to modulate key processes involved in wound healing. Moreover, previous studies have underscored its ability to influence remodelling capacity, the formation of granulation tissue, and collagen-fiber deposition, pivotal aspects of the

wound healing cascade [11]. By formulating curcumin with Vaseline and glycerin, this study aims to harness their synergistic effects, potentially enhancing curcumin's bioavailability and therapeutic efficacy. Through rigorous morphological and histological assessments, researchers seek to elucidate the specific mechanisms by which this formulation impacts cutaneous wound healing. Such insights hold promise for the development of novel wound care strategies, addressing an unmet clinical need for effective and safe interventions to accelerate wound closure and improve healing outcomes [12 - 13].

The results of the study confirm that the application of curcumin ointment on cutaneous wounds in rats significantly accelerates the healing process, similar to the effects observed with other natural substances such as Aloe Vera, which are known to improve wound healing and possess antibacterial properties [2]. The curcumin ointment formulation used in this study induced significant morphological and histological changes.

Multiple studies have indicated that applying curcumin to wounds encourages the regrowth of epithelial tissue, boosts the multiplication of fibroblasts, and enhances vascular density [13]. In this study, morphological observations revealed that the size of the wounds began to decrease as early as the 3rd day. Although, substantial improvement in skin wound healing was noted by the 12th day, the final complete disappearance of the wounds by the 14th day was observed in the Cur-group.

Histological analysis could further support these findings, showing that the density of epithelial regeneration and the amount of collagen-fibers were significantly greater in the Cur-group compared to C-group by the 7th day of treatment. These results indicate that applying curcumin facilitates both the structural and cellular aspects of wound healing, resulting in faster closure of skin wounds in rats.

In the treated group, the wound area displayed a later phase of granulation tissue formation, characterized by complete closure of the wound with minimal necrotic tissue and a scab covering the surface. This granulation tissue exhibited a disorganized arrangement of collagen fibers, appearing as small, randomly distributed fibrils, with limited angiogenesis and an intense inflammatory reaction. In comparison, by the 14th day, wounds treated with curcumin showed an early stage of the proliferative phase with improved tissue regeneration. The overlying epidermis was immature, hyperplastic, and disorganized, with a moderate inflammatory response in the dermis and hypodermis. Additionally, there was an increase in the production of the dermal layer due to the deposition of regularly arranged collagen fibres, which was accompanied by fibroblast proliferation. It

was noticed that the fibers were denser and thicker the Cur-group.

These findings are consistent with those of the previous literatures, indicating that collagen density in the Cur- group surpassed that of the C-group by day 7 and 14. This suggests an earlier and denser maturation of granulation tissue and the initiation of collagen production in the curcumin-treated wounds. These effects are attributed to curcumin molecules stimulating the production of growth factors in the wounds, which in turn accelerate the production of newly granulation, collagen fibers consistence, and the processes of remodelling and contraction [11, 14].

Curcumin exhibits low pharmaceutical features such as solubility, bioavailability, and photostability in its raw-format, thereby limiting its utility as a therapeutic agent and precautionary food support [5]. When administered orally or intraperitoneally, it shows the least absorption, and small quantities were detected in the bloodstream. However, studies have demonstrated that topical application of curcumin, when formulated with a suitable vehicle rather than in powder form, does not cause skin irritation and exhibits the highest permeability. Furthermore, topical administration of curcumin has been reported to exert a more pronounced anti-inflammatory impact on healing compared to oral administration [14 - 15].

While numerous studies have explored the beneficial impacts of curcumin on tissue healing across various regions in the different experimental trials, when administered whether intra-peritoneally, orally, and recently on surface applications, however, still there is a gap in research concerning the specific effect of topical application of curcumin combined with Vaseline and glycerin on cutaneous wound healing. Despite the existing evidence suggesting that the use of curcumin topically could enhance re-epithelialization, cell restoration, and collagen synthesis, while concurrently reducing oxygen free-radicals, tissue inflammations, and promoting wound healing, there is a lack of investigation into the combination with Vaseline and glycerin [16 - 18]. These finding, indicate the effectiveness anti-inflammatory and antioxidant properties of this prepared compound in accelerating the steps of tissue healing in the normally incised derm, and this could be applied to different types of wounds at different stages [19 - 20].

One limitation of our study is the absence of pure or high-quality curcumin, as well as the lack of a standardized cream formulation derived from curcumin.

Conclusion

In conclusion, topical application of curcumin to cutaneous wounds in rats significantly accelerates the healing process. The observed reduction in wound

healing time suggests that curcumin is a safe and effective medical agent for enhancing postoperative care, particularly in procedures involving various flaps and skin grafts. These results support the potential clinical utility of curcumin as an adjunctive therapy to promote successful wound healing following surgical interventions. More research and clinical applications are warranted to further support these findings and explore the full therapeutic potential of curcumin in wound management.

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Conflict of interest

All authors declare no conflict of interests.

Ethical approval

The procedure performed in this study was approved by the College of Veterinary Medicine Scientific Research Committee, University of Sulaimani, Kurdistan Regional Government, Kurdistan, Iraq

Statement of human and animal rights

The experimental procedures and the accession of the study were undertaken according to the principles of the Ethics Research Committee of the College of Veterinary Medicine, University of Sulaimani, Kurdistan Regional Government, Kurdistan/ Iraq.

Data Availability: Data used in this study are available from the corresponding author upon reasonable request.

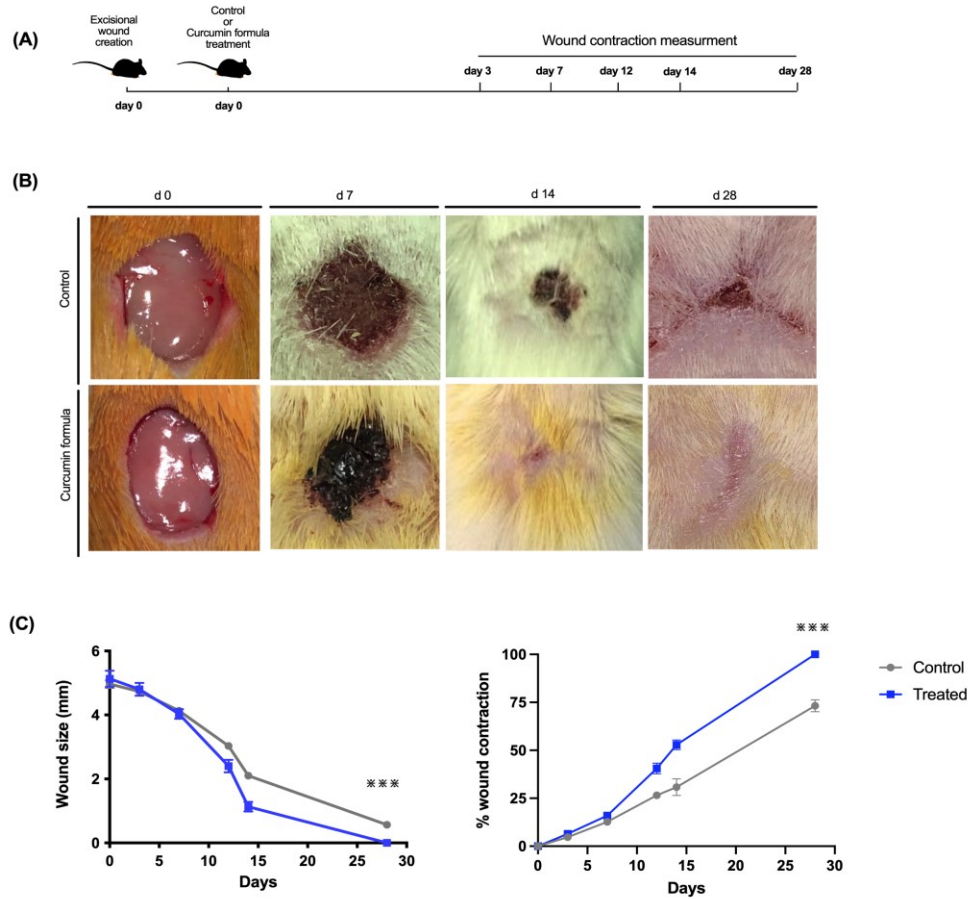


Fig. 1. (A) Experimental plan (B) Representative images show the gross morphological views of the skin wounds at days 0, 7, 14 and 28 between the control and the Cur- groups. (C) Line graphs show the wound size in mm between the control and treated groups at days 0, 3, 7, 12, 14 and 28 and the percentage of wound contraction between the two groups for the same time points. A statistically significant difference in wound contraction was found between the control and treated groups using Two-Way-ANOVA Test (***) p<0.0001), (Error bars = +/- SEM (Standard Error of Means).

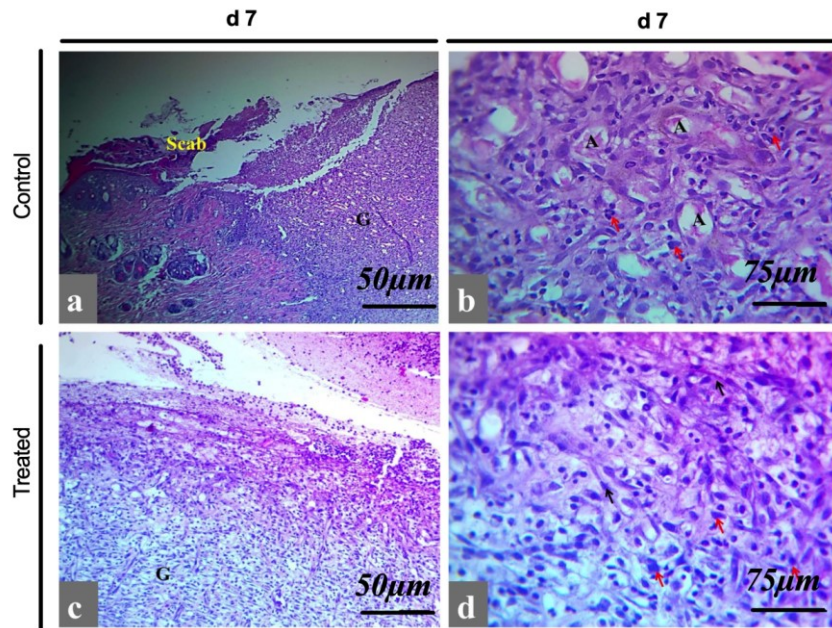


Fig. 2. Representative histological sections of the cutaneous wound at day 7. Partially closed wound in the control group that was covered by scab with vascular granulation tissue indicated by the letter G, maximum intensity angiogenesis indicated by letter A, infiltration of inflammatory cells (red arrows), c and d: A closed wound in treated group covered with loose scab tissue with intense fibro-vascular granulation indicated by letter G tissue in dermis revealed neovascularization indicated by letter A, thin and individual collagen fibers (black arrows), and intense infiltration of inflammatory cells as indicated by red arrows, (H&E stain, scale bars 75µm and 20µm).

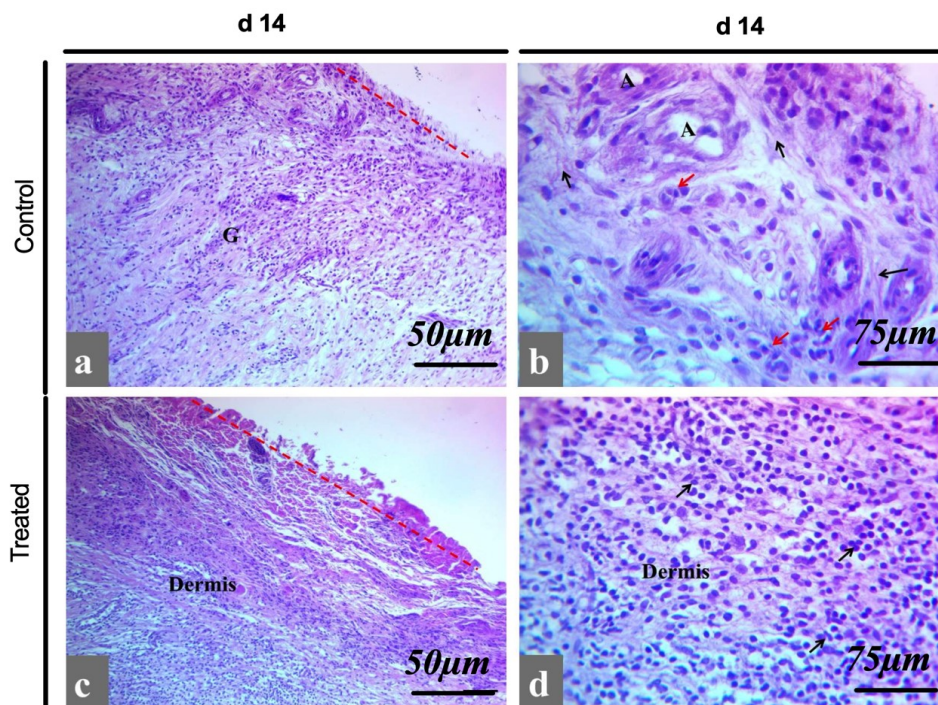


Fig. 3. Representative histological sections of the cutaneous wound at day 14. The cutaneous wound in the control group showed a thin layer of re-epithelization (red dash line) with fibro-vascular granulation tissue that showed few angiogenesis, thin and irregularly arranged collagen fibres (black arrows), and intense infiltration of inflammatory cells (indicated by red arrows), c and d: Typical hyperplastic immature disorganized epidermis for bridging the gap (red dash line), increased in thickness of dermal layer by proliferation of collagen fibres and moderate infiltration of cells as indicated by black arrows in the treated group, (H&E stain, scale bar 75µm and 20µm).

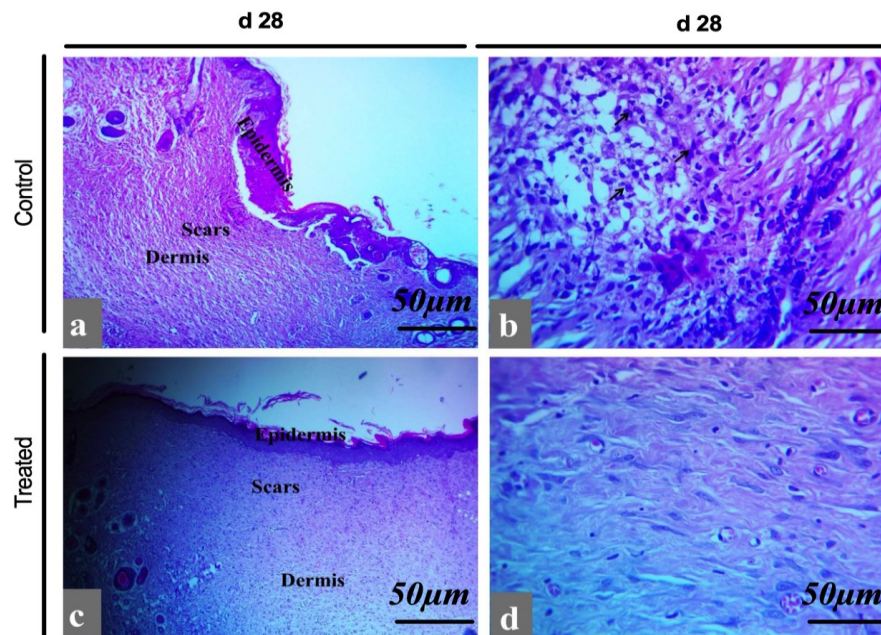


Fig 4. Representative histological sections of the cutaneous wound at day 28. Full re-epithelialization of the wound surface with uneven epidermis, thin keratinized squamous epithelium with no morphological signs of cutaneous appendages and immature scar connective tissue in the dermis shows persistence of mononuclear inflammatory cells in control group, c and d: Well-organized epidermis with thick keratinized squamous epithelium without any evidence of cutaneous adnexa and thick organized mature scar connective tissue in treated the group, (H&E stain, scale bar 75µm and 20µm).

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تعزيز التئام الجروح الجلدية: الإمكانيات العلاجية لمستخلص الكركمين الموضعي في نموذج الجرزان

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الخلاصة

تم هذه الدراسة على ٢٤ جرزان، اعتباراً ان الكركمين تعزز عملية التئام الجروح. قسمت الحيوانات إلى مجموعة مراقبة (ن = 12) ومجموعة العلاج (ن = 12). تم إنشاء جرح جلدي استئصالي بحجم 5 مم على ظهر كل جرذ وتمت علاجها بتركيبية من مرهم الكركمين موضعياً في مجموعة العلاج، بينما عولجت المجموعة التحكم بالمركبة (الفازلين والجلسرين) فقط. ونتيجة لذلك، لوحظ تحسن ملحوظ في التئام الجروح الجلدية في مجموعة العلاج بالمقارنة مع المجموعة التحكم. كان معدل تكوين الأوعية، وشدة تكوين الأنسجة الحبيبية وإعادة تكوين البشرة، وكمية ترسب ألياف الكولاجين أعلى في مجموعة العلاج مقارنة بالمجموعة التحكم. كما التطبيق الموضعي من الكركمين يعزز عملية التئام الجروح. لذلك، يمكن استخدام مرهم الكركمين كعامل طبي فعال لمنع تطور الالتهابات والأكسدة في الجرح. بناء على ذلك، فهو عامل طبي آمن وفعال يمكن استخدامه لزيادة نجاح الجراحة عندما يتم استخدامه كعناية ما بعد العملية الجراحية لتعزيز عملية تقلص الجرح والشفاء.

الكلمات المفتاحية: مرهم كركمين، تطبيق موضعي، التئام الجرح، نموذج الجرزان