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# Histopathological Assessment of Wound Healing in Mice Treated with Helianthus Tuberosus Powder

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## Abstract

**Objective: Several medicinal plants have been given significant attention in the medical field due to their effect on wound healing. This study aims to identify the effectiveness of tuberosus helianthus powder in skin wound healing in mice.**

**Material and Methods: Tuberosis helianthus powder was purchased from a commercial supplier (medicinal Korean herbs, prince herb). The powder was stored in a dry, airtight container at room temperature until use. It was applied directly to the incisional wounds at a specific concentration and frequency (50 mg twice daily). Thirty-six male mice were randomly assigned to three time points (3, 7, and 14 days), with 12 mice per time point. Each mouse received two incisional wounds: one on the left side, serving as the control, and another on the right side, designated as the experimental wound. At each time point, 12 mice were euthanized, yielding 24 wound samples (12 control and 12 experimental) per time point. In total, 36 control wounds and 36 experimental wounds were analyzed. Three physical parameters were taken, including the number of inflammatory cells, blood vessels, and epidermis thickness. All these parameters were on the dorsal aspect of the thoracolumbar region, into which tuberous helianthus powder was typically done in the treated day duration. Meanwhile, the day durations for control mice were left without any treatment. The mice were euthanized on days 3, 7, and 14 after wound healing for histopathological study.**

**Results: The histopathological analysis using hematoxylin and eosin (H&E) staining revealed significant differences between the control and treatment groups. On day 3, the control group exhibited pronounced inflammatory responses with extensive inflammatory cell infiltration, necrotic tissue, and fibrin clot formation. In contrast, the treatment group showed early signs of wound healing, such as mild inflammatory response and reepithelialization with keratinized stratified squamous epithelium. Throughout the study, the treatment group demonstrated faster wound contraction, enhanced tissue remodeling, and reduced inflammatory cells compared to the control. By day 14, treated mice displayed mature epidermis and dermal granulation tissue with significant angiogenesis, suggesting accelerated wound healing. These findings support the potential of Helianthus tuberosus powder in promoting wound healing.**

**Conclusion: Tuberosus helianthus powder plays an important role in the healing pathway in wound healing.**

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## Introduction

The healing of skin wounds is a problematic biological procedure by which skin repairs itself after a wound [1,2]. Wound healing is a natural process that returns the skin or mucosa to its normal state [3]. This procedure comprises three highly combined stages: Inflammation, tissue proliferation, and remodeling [4]. It includes the activation of inflammatory cells, endothelial cells, keratinocytes, and fibroblasts [5], along with the mixture and liberation of active mediators (cytokines and development parameters) to adjust tissue repair and recreation [2,6]. These stages and their physiological parts might happen in the suitable series and at certain times to realize the ideal function and the flexible strength of the wounded position of the source skin [6]. Different methods are utilized to support skin wound healing, such as managing local or systemic antibiotics or using antiseptic results [7]. The increasing of opposing pathogenic against presently utilized simulated medications managers like antifungal [8], antiviral products and antibiotics has encouraged interest among several investigators in the determination of novel antibacterial combinations created from plants [9], plant depend on phytochemicals as potential alternatives to antibiotics that are still utilized in modern and classical schemes of drug for the disease treatment [10]. The existence of multiple life-sustaining constituents in plants has encouraged researchers to concentrate on plants for their extensive healing wounds potential [11] .Currently, plants or chemical combinations derived from plants have been utilized as pharmaceutical product managers for treating skin wounds [12]. Medicinal plants show a vital key in wound healing via several different mechanisms like reducing the bacterial amount, improving angiogenesis, rushing the deposition of collagen, and rising fibroblast proliferation [13].

The biological features of medicinal plants affect key actions in mammalian wound healing, displaying a vital character in regulating the wound-healing pathway [14]. The knowledge of the medicinal value of plants and other substances and their use goes back to the earliest settlers [15]. They are hence regarded as a basis of several macromolecules like fatty acids, carbohydrates, and proteins [16], which can be utilized as biomaterials for emerging new candidate medications to support wound healing [17]. Around 500,000 plant types exist worldwide, and only 1% have been investigated [18].

Tuberosus helianthus is a sunflower that appeared in eastern North America and is extensively distributed in the Middle East, particularly Iraq [19]. This plant is (2–3 mm) long with superficial leaves and plump tubers [20]. As the plant was civilized by Native Americans first, they denoted the plant as sun roots. While the plant was presented to other portions of the world [21], varied standard terms were attributed in several languages [22]. It has been recognized that this type shows several medical activities, such as diuretic, purgative, and bowel tonic impacts [23]. It has also been utilized as a folk medicine for treating bone fractures and cutaneous wounds, and even for releasing pain [24]. Studies have also shown that tuberous helianthus combinations have anti-inflammatory, antimicrobial, antioxidant, antifungal, anticancer, antipyretic, and analgesic impacts [25].

Tuberosus helianthus is rich in fatty acids like plasmatic acid that resemble those current in the sebaceous glands in the skin [26]. These fatty acids have been recorded to keep strong antimicrobial–disinfectant features in the skin [27]. This plant has been utilized generally as a dietary supplement for pain management, to decrease swelling [23], to increase the immune system, and to treat skin wounds in general medicine. It is an abundant source of bioactive compounds like phenolic acids, coumarins [28], and flavonoids that are recognized to use pharmacological actions, comprising anti-inflammatory, antimicrobial, and antioxidant features [29].

This study aims to identify the effectiveness of tuberosus helianthus powder on skin wound healing in mice.

## Material and Methods

This study was cross-sectional and approved by Baghdad University, Baghdad Dental College, Iraq—thirty-six adult male mice from April 2024 to July 2024. The mice used in the experiment protocol were from Baghdad University Baghdad Dental College. The samples were treated at the Laboratories of the Animal House Center in the College of Baghdad Medicine, Baghdad University. Thirty-six adult male mice will be subjected to the induced wound on the right dorsal region of each mouse. A- Incisional wound on the left side of the dorsal region will be treated daily with normal saline for three different durations (3, 7 and 14 days). B-Experimental group: 36 mice will be subjected to the induced incisional wound on the right side of the dorsal region of the mice and will be treated daily with tuberosus helianthus powder for three different day durations (3, 7 and 14 days).

A full thickness wound1cm×1 cm in size was established on the right side of the dorsal aspect of the animal, into which tuberosus helianthus powder was topically applied in the treated group. In contrast, the control group was left without any treatment. All samples were excised and fixed in 10% formalin. After fixation, they were processed; block and slides were prepared and then stained in Hematoxylin / Eosin for histological studies [30].

**Preparation of tuberous helianthus powder**: Tuberosus helianthus powder was purchased from a commercial supplier (medicinal Korean herbs, prince herb). The powder was stored in a dry, airtight container at room temperature until use [31]. It was applied directly to the incisional wounds at a specific concentration and frequency (50 mg twice daily). Using Helianthus tuberosus in powder form instead of liquid or other preparations can have several advantages in wound healing, like Stability and shelf Life, Controlled Dosage, Sustained Release, Direct Application and Enhanced Absorption and coverage [32]. 36 adult male mice were classified into experimental and control groups (one incision on the left side -the control and one on the right side experimental), with three different day durations (3, 7 and 14 days). Three physical parameters were taken, like number of inflammatory cells, blood vessels, and epidermis thickness. All these parameters were on the dorsal aspect of the thoracolumbar region, into which tuberous helianthus powder was topically done in the treated day duration. The day durations for the control incision were left without any treatment. The mice were euthanized on days 3, 7, and 14 after wound healing for histopathological study.

**Animals**: Thirty-six male young adults (7-8 weeks old) Kunming mice weighing 20-25 g were purchased from the animal house at the College of Veterinary Medicine, Baghdad University. The environment in the cages was kept at room temperature of 25 ± 2°C [33], humidity 55-60%, with a 12:12 h light/dark cycle. The animals were fed standard pellets and tap water. We used male mice in this study to reduce the contribution of hormonal variation associated with the oestrous cycle of female mice, which can affect inflammatory responses and tissue remodeling in wound healing [34].

**Wound creation**: General anaesthesia was performed with a mixture of 90 mg/kg ketamine (Alfasan, Holland) and 10 mg/kg xylazine (Rugby-Virbac Laboratories, France) intraperitoneally. The dorsal part of the thoracolumbar area on both sides was surgically prepared, and full-thickness 1 cm×1 cm wounds were created with a surgical scalpel (blade No.11) on all the animals. Surgery was performed under a sterile condition [35]. The lesions on the left side were left undressed overnight to the environment to grow, Microbial

colonization and proliferation. In contrast, the right side was treated with the powder, and 50 mg of H. tuberosus powder was topically applied twice daily for the injury. The incisional wound was made because it enables precise evaluation of inflammation, fibroblast activity, and collagen remodeling. It is a reliable and reproducible method of measuring wound closure over time. Also, it simulates surgical wounds, which makes it pertinent for possible Helianthus tuberosus clinical applications, such as post-surgical healing [36].

**Sample size calculations:** The sample size calculation was done utilizing statistical analyses achieved using the statistical package for social sciences (SPSS) ver. 29 with Microsoft Excel 2021. Results of the complete number of mice 36 adult male mice from April 2024 to July 2024. The α error was 0.05, and the power was 0.90 for sample size calculation. Frequency distribution for nominated variables describing the detailed mice with samples was done. The entire test was commonly distributed, as variables are presented by different statistical parameters like mean, standard deviation (SD), and standard error mean. A one-way ANOVA test was used to compare the experimental and control groups for three days, shown at a significance level of 0.05.

## Results

This study was cross-sectional and approved by Baghdad University, Baghdad Dental College, Iraq. Thirty-six adult male mice from April 2024 to July 2024. All mice will be subjected to a traumatic incision of 1^1cm, with a surgical blade (no 11) on the right side of the dorsum for each mouse with the application of tuberous helianthus powder on the wound. The mice were euthanized on days 3, 7, and 14 days after the wound, making for a histopathological study.

Using ImageJ software, the histopathological analysis will be achieved on hematoxylin and eosin-stained slides, including the inflammatory cells, epithelial thickness, and blood vessel numbers.

**Macroscopic Assessment of Wound Healing**: Wound contraction over time was macroscopically evaluated as the healing process of the incisional wounds. Digital photographs of these wounds were acquired at multiple time points (D3, D7, and D14) for both groups as visual estimates of wound closure. In addition, , wound contraction was quantified by a vernier caliper to evaluate the decreasing surface area of wounds with time.

Wound healing was noticeably enhanced in Helianthus tuberosus powder-treated animals compared to untreated animals.

Dressed wounds showed earlier signs of closure with lower levels of inflammation and higher reductions in wound size over time. In contrast, the control group displayed impaired healing with an increased wound size and prolonged inflammation.

These results concluded that Helianthus tuberosus powder could improve wounds' healing response, leading to faster contraction and lower inflammatory responses in the early days. This observation is further supported by representative images at various time points of wound closure in both groups (Figure 1).

**Microscopic assessment of wound healing-Histopathological result (Hematoxylin & eosin) stain:** Figures A through N summarize microscopic assessements.

**Effect of the number of inflammatory cells results on all day durations:** A one-way ANOVA test is used to analyze the effect of the number of inflammatory cells on all-day durations. A p-value >0.05 indicates no significant differences between the number of inflammatory cells results and all-day durations. At the same time, P-Value < 0.05 represents the significant differences between the number of inflammatory cells and all-day durations. There was a highly significant difference in the number of inflammatory cells results among all day durations according to P-value=0.000. It can be observed that the highest mean value (98.00) of 3 days of control is attributed to the number of inflammatory cells at this duration being very high compared with other day durations. The lowest standard deviation value (0.707) of 14 days (control negative) was due to a slight difference in values among all the inflammatory cells at this duration, as displayed in Table 1.

**Effect of the number of blood vessels results on all day durations:** A one-way ANOVA test is used to analyze the effect of the number of blood vessel results according to all-day durations. There was a highly significant difference between the numbers of blood vessel results and all-day durations concerning P-value=0.000, as displayed in Table 2. It can be observed that the maximum mean value (8.20) of 7 days post-treatment is attributed to the number of blood vessels at this duration, which is very high compared to other day durations. The minimum standard deviation value (0.447) of 14-day durations post-treatment is due to a slight difference in values among the number of blood vessels at this duration.

**Effect of the thickness of epidermis results on all day durations**: A one-way ANOVA test was used to analyze the effect of the thickness of epidermis results according to all-day durations. There was a highly significant difference between the thickness of epidermis results and all-day durations concerning P-value=0.000, as exhibited in Table 3. It can be clearly shown that the maximum mean value (82.1164) of 3-day durations post-treatment is attributed to the thickness of the epidermis at this duration being very high compared with other day durations. The minimum standard deviation value (0.5254) of 14-day durations (control negative) is due to a slight value difference among all the epidermis thicknesses at this duration. Furthermore, there was no formation around the tissue at this duration.

## Discussion

This study evaluated the histopathological aspects of the treatment of wound healing, emphasizing inflammatory cell infiltration, vascularization, epidermal thickness and maturity of the connective tissue. The results confirmed significant differences between experimental groups at different time points using one-way ANOVA followed by post hoc tests, emphasizing the efficacy of the tested treatment in enhancing the wound healing process [37]. However, on day 3, the treated group had significantly fewer inflammatory cells (mean = 32.40), indicating that the treatment helped reduce the early inflammatory response. Controlled inflammation is essential for wound healing; however, it should not be excessive, since it leads to delayed wound healing [38].

Additionally, the reductions in inflammatory cell counts observed in the post-treatment groups persisted with time. On day 14, the treated group had the least inflammatory cells (mean = 1.80), suggesting a reduced inflammatory response. P < 0.05, highlighting the promise of treatment to drive the less inflammatory environment required for wound healing [39].

**Vascularization:** Angiogenesis—forming new blood vessels—is a key component of wound healing. The data were analysed statistically, and a marked increase in the number of blood vessels in the treated groups vs the control groups was seen (O/L). The treated group had a mean of 8.20 blood vessels per HPF (p<0.05) compared to the 6.40 in the control on day 7 post-treatment. Indeed, the treatment-generated difference (p < 0.05) suggests that angiogenesis was encouraged and allows for the delivery of oxygen and nutrients to the regenerating tissue, as well as transitioning the healing process from inflammatory to proliferative [38]. These results also validated the favorable effect of the treatment on vascularization, which would be shown by improvements in the vascular content of the treated groups over the other groups at later time points (days 7 and 14).

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**Epidermal Thickness**: Epidermal thickness is a critical indicator of epithelialization, and our results show a significant increase in epidermal thickness in the post-treatment groups. On day 3 post-treatment, the treated group had an average epidermal thickness of 82.12 µm, significantly more significant than the control group, which showed a fibrin clot and necrotic tissue in the epidermal layer. The above difference ($p<0.05$) confirms that the treatment resulted in a faster reepithelization and a thicker epidermal sheet with stronger morphology(40). The treated group still had epithelial messaging by day 14, emphasizing how EGF treatment accelerated wound healing and induced epidermal integrity.

**Maturation of Connective Tissue:** Another very important factor of wound healing is the maturation of connective tissue. Connective tissue type analysis showed significant differences in inflammatory cell numbers about each type. The control group at day 3 showed necrotic tissue with many inflammatory cells (mean = 98.00), while the treated groups exhibited more mature granulation tissue intestinal types. Statistical analysis suggested that necrotic connective tissue was significantly associated with more significant inflammatory cell counts than other tissue types, including immature granulation tissue and mature granulation tissue. These had the combined effect of enhancing the shift from necrotic tissue to more granulation tissue, which is crucial in the remodeling phase of wound healing [41].

## Conclusion

Wound healing is a complex process that involves inflammation, granulation and tissue remodeling. Interactions of different cells, extracellular matrix proteins and their receptors are involved in wound healing and are mediated by cytokines and growth factors [42]. The statistical analysis of the histopathological data corroborates the fact that the treatment dramatically accelerates the healing process. The decrease in inflammatory cells, increased vascularity, thickened epidermal layer, and collagen maturation suggest that treatment acts on multiple parts of the wound healing cascade. These data corroborate the literature on non-invasive therapeutic strategies for regenerative enhancement of cutaneous wound healing [38]. Overall, since the treatment reduced inflammation, promoted angiogenesis, and accelerated healing of the epithelial and connective tissues, it may be an effective therapeutic strategy in wound healing.

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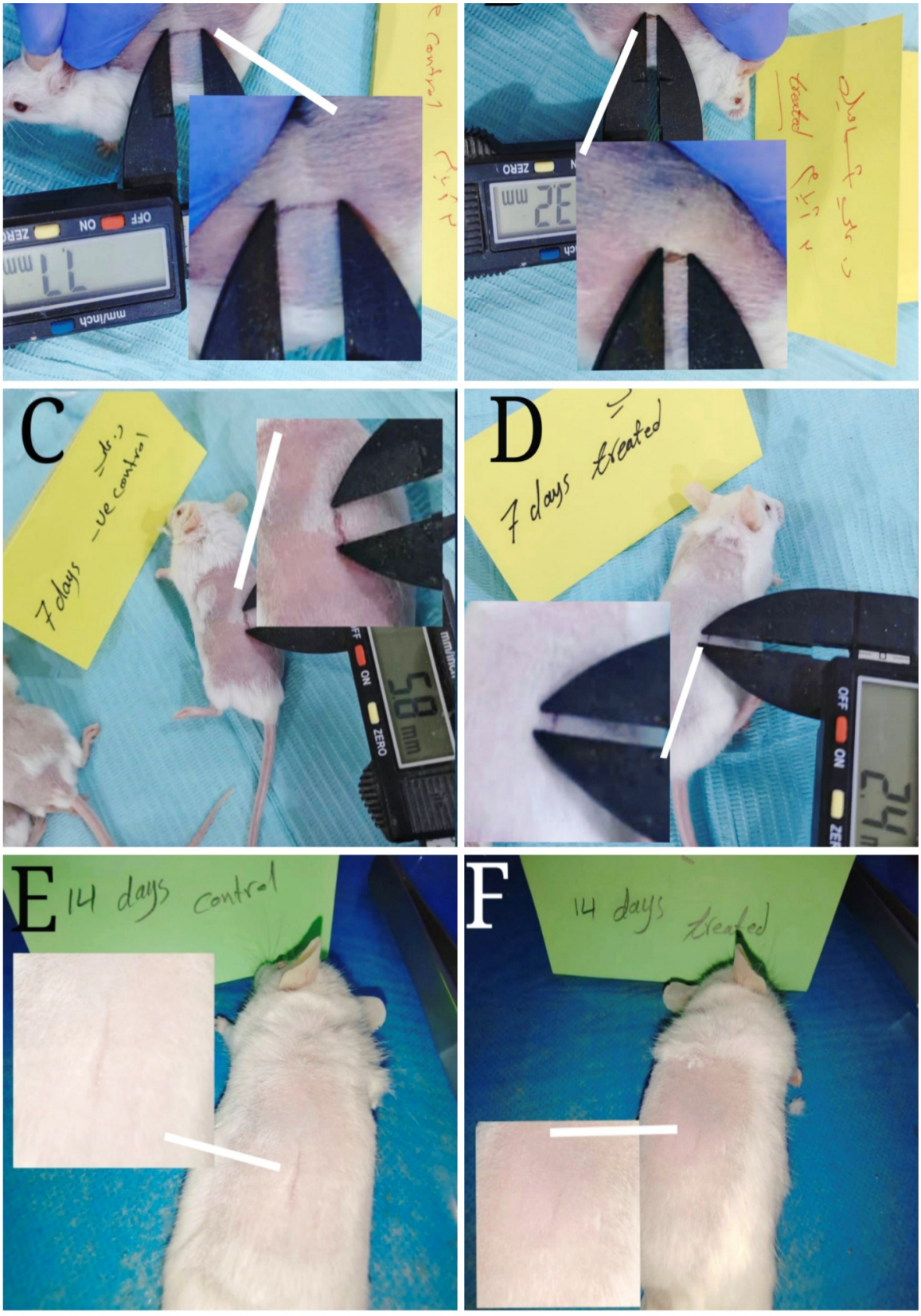
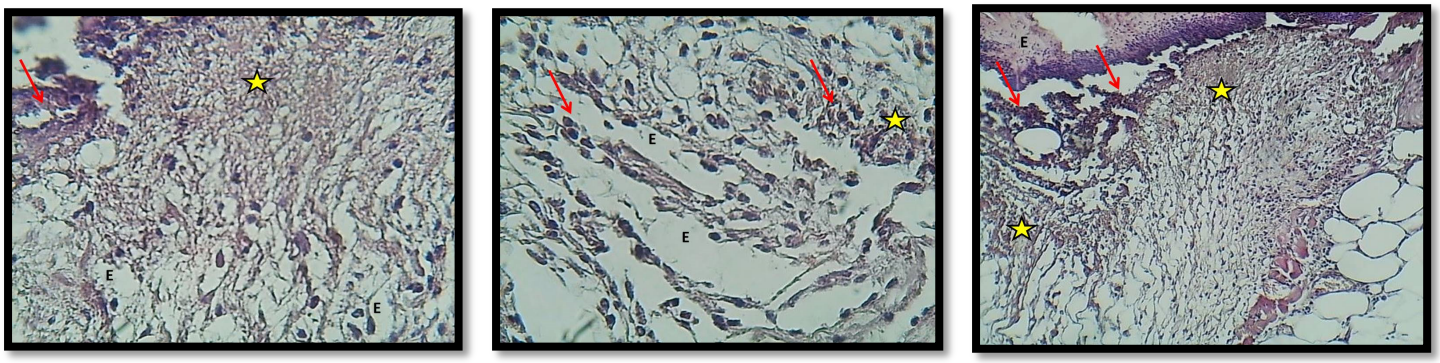



Figure 1. Macroscopic images of the wound contraction in the treated and control groups. (A) Day 3 control. (B) Day 3 treated. (C) Day 7 control. (D) Day 7 treated. (E) Day 14 control. (F) Day 14 treated.

**3 days (control negative)**

The histopathological figures of the skin (control negative) showed marked dermatitis that characterized by marked loss of the epidermis that replaced with thick layer of fibrin clot followed by thick line of necrotic tissue with marked infiltration of inflammatory cells (MNCs) (A and B), the dermis revealed dermatitis with severe degeneration and necrosis of dermal collagen fibers, severe infiltration of leukocytes and edema (C).

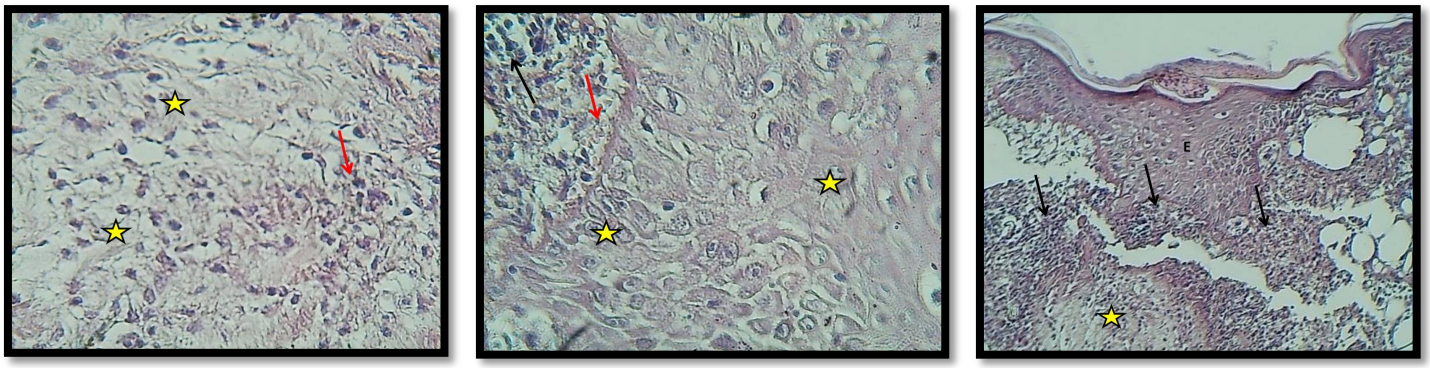
A B C




**3 days (post- treatment)**

The histopathological figures of the skin dermis revealed marked reepithelization (Proliferation of epithelial cells) and formation of keratinized stratified squamous epithelium (D). There were thin line of necrotic tissue with mild infiltration of mono nuclear leukocytes (MNCs) mainly lymphocytes with little hemorrhage between dermis and epidermis (D and E). The dermis comprised of immature fibrous connective tissue with furthermore fibroblasts and no hair follicles or sebaceous glands (F).

D E F



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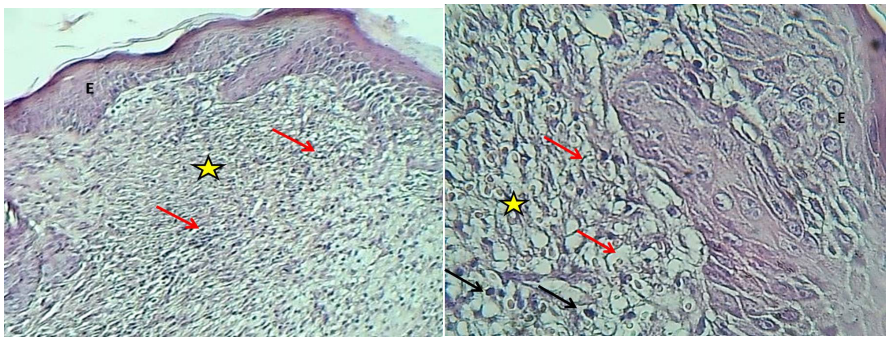
**7 days (control negative)**

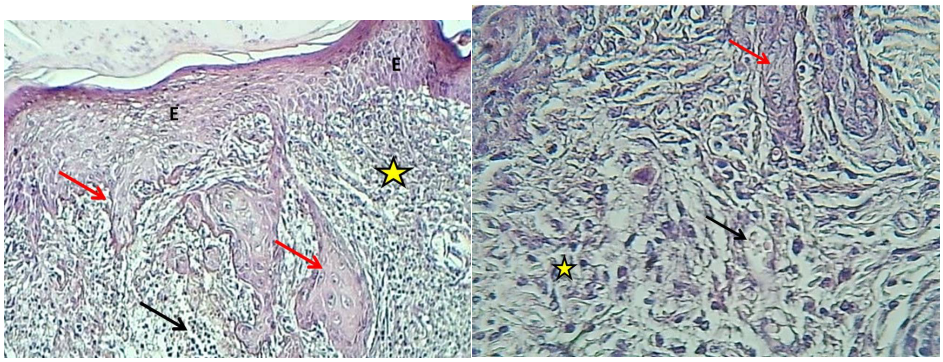
The histopathological figures of the skin (control negative) showed proliferating epithelial cells of epidermis (stratified squamous epithelium (G). The dermis revealed immature fibrous connective tissue with marked meshwork of angiogenesis (newly formed blood vessel) and moderate infiltration of leukocytes, the figures revealed no sebaceous glands or hair follicles (H).




G H

**7 days post treatment**

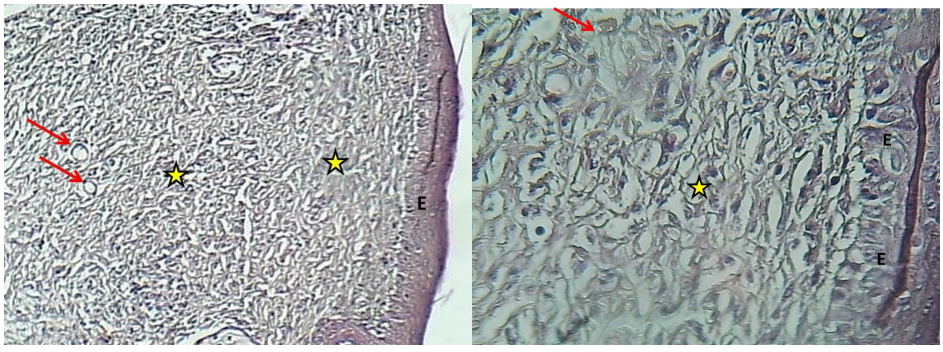
The histopathological figures of the skin revealed marked epidermal hyperplasia that characterized by formation of epidermal papilla (I). The dermis comprised of slightly mature granulation tissue with little infiltration of leukocytes and marked angiogenesis. The figures revealed nor hair follicles and neither sebaceous glands (J).




I J

**14 days (control negative)**

The histopathological figures of the skin (control negative) showed mature epithelial cells of epidermis (stratified squamous epithelium) (K). The dermis revealed slightly mature granulation tissue with many newly formed blood vessels, there was no infiltration of leukocytes and the figures revealed no sebaceous glands or hair follicles (L).




K L

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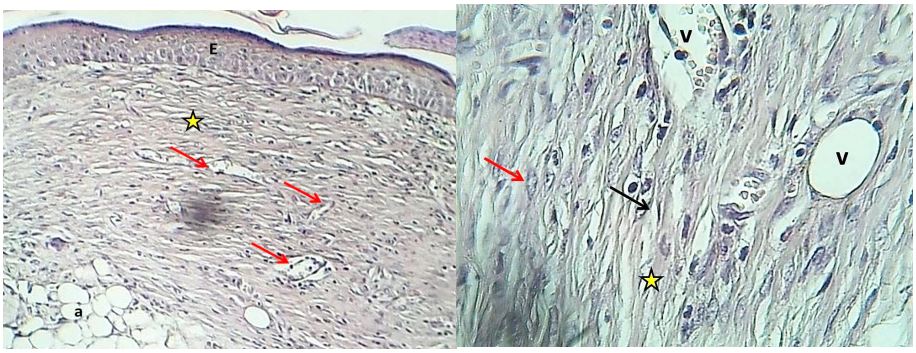
**14 days post treatment**

The histopathological figures of the skin revealed mature epidermis epithelial cells (M). The dermis comprised of mature granulation tissue with marked angiogenesis (numerous blood vessels). The figures revealed no hair follicles and neither sebaceous glands and there is normal adipose tissue of hypodermis (N).




M N

Table 1. The relative frequency of the number of inflammatory cells in each duration using the one-way ANOVA test.

| No. of inflammatory cells | | | | | |
|---|---|---|---|---|---|
| **Day durations** | **N** | **Mean** | **Std. Deviation** | **Std. Error** | **P-value** |
| 3 days-control | 5 | 98.00 | 5.385 | 2.408 | 0.000 |
| 3 days post-treatment | 5 | 32.40 | 6.229 | 2.786 | |
| 7 days-control | 5 | 19.60 | 3.362 | 1.503 | |
| 7 days-post treatment | 5 | 11.60 | 1.517 | .678 | |
| 14 days-control | 5 | 3.00 | .707 | .316 | |
| 14 days-post treatment | 5 | 1.80 | .837 | .374 | |
| Total | 30 | 27.73 | 33.825 | 6.176 | |

Table 2. The relative frequency of the number of blood vessels results in each duration using the one-way ANOVA test.

| No. of blood vessels | | | | | |
|---|---|---|---|---|---|
| **Day durations** | **N** | **Mean** | **Standard Deviation** | **Standard Error** | **P-value** |
| 3 days-control | 5 | 1.00 | 1.000 | .447 | 0.000 |
| 3 days post-treatment | 5 | 4.00 | 1.225 | .548 | |
| 7 days-control | 5 | 6.40 | .894 | .400 | |
| 7 days-post treatment | 5 | 8.20 | 1.095 | .490 | |
| 14 days-control | 5 | 1.80 | .837 | .374 | |
| 14 days-post treatment | 5 | 3.20 | .447 | .200 | |
| Total | 30 | 4.10 | 2.695 | .492 | |

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Table 3. The relative frequency of the thickness of the epidermis results in each duration using the one-way ANOVA test.

| Thickness of epidermis | | | | | |
|---|---|---|---|---|---|
| **Day durations** | N | Mean | Standard Deviation | Standard Error | P-value |
| 3 days-control | 5 | .00000 | .0000 | .0000 | 0.000 |
| 3 days post-treatment | 5 | 82.1164 | 3.1397 | 1.4041 | |
| 7 days-control | 5 | 43.8006 | 2.4992 | 1.1177 | |
| 7 days post-treatment | 5 | 34.6008 | 1.9686 | .8803 | |
| 14 days-control | 5 | 25.8884 | .5254 | .2350 | |
| 14 days post-treatment | 5 | 26.5034 | 1.9070 | .8528 | |
| Total | 30 | 35.4849 | 25.2418 | 4.6085 | |