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Histological Evaluation of Ficus carica Leaf Extracts on Skin Wound Healing in Oryctolagus cuniculus

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***Abstract*—** Wound healing aims to restore the integrity and function of the skin. This process occurs through overlapping phases including inflammation, cell proliferation, and tissue remodeling. The present study aimed to evaluate the effect of fig leaf extract (Ficus carica) in accelerating skin wound healing by examining histological changes during different healing stages compared with a control group. Sixteen adult male rabbits were used in this study and divided into two groups (n = 8 each) at random: a control group and a group treated with Ficus carica. Standardized induced wounds were treated topically with the plant extract.

When compared to the control group, the treated group's wound healing significantly improved, according to the data. Fig leaf extract decreased bleeding, enhanced collagen fiber organization and granulation tissue production, and sped up wound healing and epidermal regeneration. The efficiency of fig leaf extract in promoting wound healing was demonstrated by statistical analysis, which showed significant differences ($P < 0.05$) between the two groups in the majority of histological criteria. According to these results, fig leaf extract may help restore normal skin architecture by speeding up wound healing and enhancing tissue remodeling.

Keywords: Wound healing, Ficus carica, tissue remodeling**.**

INTRODUCTION

Skin is the largest organ in the body and is considered the primary line of defense of the integumentary system. It performs many vital functions in maintaining body homeostasis, including thermoregulation, hydration regulation, immunological defense, sensory perception, and vitamin D production. The skin also acts as a physical barrier against environmental hazards such as toxins, viruses, and ultraviolet radiation (1). Structurally, the skin is composed of the dermis and epidermis, which are supported by subcutaneous tissues. Coordinated interactions between these layers are essential for both tissue repair and proper physiological function (2,3).

Wound healing is a complex and dynamic biological process consisting of several overlapping phases, including hemostasis, inflammation, proliferation, and remodeling. During these phases, inflammatory cells, fibroblasts, keratinocytes, and extracellular matrix components must coordinate precisely to restore tissue integrity (1,4). Both the epidermis and dermis sustain extensive injury in full-thickness skin wounds, and due to the limited regenerative capacity of the dermis, healing often results in scar formation rather than complete tissue regeneration (5).

Fibroblasts play a crucial role in the production of collagen and extracellular matrix during the healing process, both of which are necessary for rebuilding the strength and structure of the skin. Collagen deposition is an essential step in tissue repair. Initially, collagen deposition forms a temporary matrix, which is later replaced by the stronger type I collagen during the remodeling phase (6). The final outcomes of wound healing, including tensile strength and scar appearance, are greatly influenced by the quality of collagen organization.

Plant-based therapies are considered promising because they are more available, less expensive, and have fewer side effects compared with synthetic drugs. These natural products contain bioactive compounds such as flavonoids, phenolic acids, tannins, and saponins, which possess antibacterial, anti-inflammatory, and antioxidant properties (7,8). These compounds can positively influence several stages of wound healing by reducing oxidative stress, regulating inflammation, and increasing fibroblast activity and collagen formation.

Ficus carica (fig) is one of the medicinal plants that has attracted considerable interest because of its wide range of pharmacological activities and rich phytochemical composition. Fig leaves contain bioactive compounds including flavonoids (rutin and quercetin), phenolic acids, coumarins, and tannins, which contribute to their therapeutic properties (9,10). These compounds are known to possess strong antioxidant and anti-inflammatory properties, which are important in regulating wound healing. Research has shown that fig leaf extract can enhance tissue regeneration by improving cellular repair pathways and regulating inflammatory mediators (11,12).

However, the histological effects of fig leaf extract during the various phases of skin wound healing are still not well understood. To evaluate the histological effect of Ficus carica

leaf extract on skin wound healing in rabbits compared with a control group, the present study was conducted.

MATERIALS AND MTHODS

Experimental design:

This study was conducted in a private garden on September 1, 2025. Sixteen healthy male rabbits, aged six months, were obtained from the animal facility of Al-Ataba Al-Husseiniya Nurseries, Al-Hafidh District, Karbala, Iraq. The animals were randomly divided into two equal groups (n = 8 per group):

- **Group A:** Control group treated with normal physiological saline.
- **Group C:** Rabbits treated with *Ficus carica* (fig) leaf extract ointment.

The experiment lasted approximately one and a half months. During the study period, rabbits were housed individually in cages (60 × 45 × 45 cm) under standardized environmental and hygienic conditions, with free access to commercial feed and clean drinking water.

Preparation of Experimental Animals

The rabbits weighed between 1.5 and 2.0 kg and were acclimatized for two weeks before the experiment. A clinical examination was performed to ensure that all animals were healthy and free from any visible skin lesions or diseases before inclusion in the study.

Housing and Feeding Conditions

Rabbits were housed individually in cages measuring 60 × 45 × 45 cm under uniform hygienic and environmental conditions with adequate ventilation and regular cleaning. Animals were maintained at room temperature under a natural light–dark cycle and were provided with a standard commercial rabbit diet and clean drinking water ad libitum throughout the experimental period.

Preparation of Fig Leaf Extract

Fresh fig (*Ficus carica*) leaves were collected from healthy trees grown in orchards in Al-Husseiniyah, Karbala, Iraq. The leaves were washed thoroughly with running water to remove dust and debris and then shade-dried at room temperature until completely dry.

The dried leaves were ground into a fine powder using a laboratory grinder. Fifty grams of the powder were mixed with 500 mL of 70% ethanol and left to macerate at room temperature for 48 h with gentle daily stirring.

The extract was filtered twice through cotton cloth and filter paper to remove plant residues. The filtrate was collected in dark glass bottles and stored at 4°C. Ethanol was removed using a water bath under reduced pressure at 45°C to obtain the crude extract.

The extraction yield was calculated according to the following equation:

Yield (%) = (Weight of dried extract / Weight of initial plant material) × 100

Yield (%) = (7 g / 50 g) × 100 = 14%

Based on the extraction yield, the final concentration of fig leaf extract in the ointment was adjusted to 7% (w/w).

The ointment consisted of white petrolatum (84%), glycerin (6%), sunflower oil (5%), and fig leaf extract (7%). White petrolatum was used as the ointment base because of its moisture-retaining properties and suitability for topical formulations (13). Glycerin was included as a humectant and co-solvent to improve spreadability and skin hydration (14). Sunflower oil served as an emollient and carrier to facilitate uniform distribution of the extract throughout the ointment base (15).

Preparation Method

White petrolatum was melted in a water bath at 40°C. Glycerin and sunflower oil were mixed thoroughly, followed by gradual incorporation of the fig leaf extract to obtain a homogeneous paste. The mixture was slowly added to the melted petrolatum with continuous stirring until a uniform ointment was obtained. The final ointment was transferred into sterile containers and stored at room temperature away from light and moisture until use (16).

Anesthesia and Experimental Wound Creation

All surgical procedures were performed at the Surgical Unit, College of Veterinary Medicine, University of Karbala, according to accepted aseptic surgical techniques and ethical guidelines for animal experimentation (17).

A square full-thickness skin wound measuring 20 × 20 mm was created on the dorsal region of each rabbit. Standardization of wound size was performed to minimize variability among animals (18).

Animals were anesthetized intramuscularly with ketamine (40 mg/kg) and xylazine (5 mg/kg). This anesthetic combination is widely used in laboratory animals to provide adequate sedation and analgesia (17). After shaving and disinfecting the dorsal area, a full-thickness wound was created using sterile surgical instruments.

Sample Collection

Wound healing was monitored clinically using a Vernier caliper, and wound measurements were recorded at predetermined intervals to calculate wound closure percentages (18).

Skin tissue samples were collected from both the wound margins and center under aseptic conditions at selected healing periods. Samples were washed with sterile saline and fixed in 10% neutral buffered formalin for histological examination.

Histological Examination

Histological evaluation was performed to assess tissue regeneration, inflammatory response, collagen deposition, and structural changes during wound healing.

Hematoxylin and Eosin (H&E) Staining

Hematoxylin and eosin staining was used to evaluate the general histological architecture of the skin. Hematoxylin stains nuclei blue-purple, whereas eosin stains the cytoplasm and extracellular matrix pink, allowing assessment of cellular and structural changes during wound healing (19).

Masson's Trichrome Staining

Masson's trichrome stain was used to evaluate collagen deposition and connective tissue remodeling. Collagen fibers appeared blue or green, muscle fibers red, and nuclei darkly stained, facilitating assessment of wound maturation and tissue organization.

Light Microscopy

Histological sections were examined and photographed using a light microscope equipped with ×10 and ×40 objective

lenses and a ×10 eyepiece, providing final magnifications of ×100 and ×400, respectively.

Statistical Analysis

Data were expressed as Mean ± Standard Error (Mean ± SE), with five samples analyzed per group (n = 5). Statistical differences among groups were evaluated using one-way ANOVA followed by Tukey's post hoc test. A value of $P < 0.05$ was considered statistically significant, and different letters (a, b, c) were used to indicate significant differences among groups.

RESULT AND DISCUSSION

At a significance level of ($p < 0.05$), the results showed distinct statistically significant differences in the thickness of the skin layers (epidermis, dermis, and hypodermis) between the control group and the group treated with Ficus carica extract throughout the time points under investigation (days 3,7,14, and 21). These time periods were chosen to mirror the major biological stages of wound healing, which include the proliferative phase, the inflammatory phase, and tissue remodeling. This enables a more precise assessment of the therapeutic impact throughout the healing process (4).

On day 3, the treatment group's epidermis was much thicker than the control group's ($p < 0.05$), suggesting an early acceleration of re-epithelialization. The bioactive substances found in Ficus carica, especially flavonoids and phenolic components, which are well-known for their potent anti-inflammatory and antioxidant properties, are responsible for this early reaction (1).

On day 7, the Ficus carica treated group showed a significant reduction in inflammatory infiltration and an increase in fibroblast activity compared to the control group ($p < 0.05$), indicating an accelerated transition from the inflammatory phase to the proliferative phase and improved granulation tissue formation.

This effect can be attributed to the antioxidant and anti-inflammatory properties of the extract, which reduce oxidative stress at the wound site and promote fibroblast activation and early extracellular matrix deposition, thereby enhancing the efficiency of tissue repair (4,20).

These substances probably promote quicker surface closure by lowering oxidative stress at the wound site and improving the conditions for keratinocyte migration and proliferation. There was no statistically significant difference between the two groups by day 14 ($p > 0.05$), which might mean that both groups had evolved to a more advanced stage of epithelial covering, when differences become less noticeable as healing gets closer to completion (20).

On day 21, however, the treated group once more had a substantial increase in epidermal thickness ($p < 0.05$), indicating that the extract may help enhance the maturation and structural organization of the newly produced epidermis in addition to accelerating early closure. On day three, the treatment group's dermis was much thicker than the control group's ($p < 0.05$), indicating a quicker initiation of granulation tissue production and an earlier activation of fibroblasts (4).

This suggests that the inflammatory phase is giving way to the proliferative phase more quickly. On day 14, the impact was still substantial ($p < 0.05$), indicating better extracellular matrix (ECM) remodeling and increased collagen deposition, both of which are critical for regaining tissue integrity and strength (21، 4). However, by day 21, there was no discernible difference between the groups ($p > 0.05$), indicating that both groups' remodeling phases had matured to a comparable degree, which is consistent with the latter stages' natural convergence of healing results (20).

Throughout the course of the trial, the Ficus carica-treated group's hypodermal layer thickness gradually increased in comparison to the control group; significant differences were noted at certain time points ($p < 0.05$). This improvement most likely results from improved structural component preservation, decreased inflammatory damage in deeper tissues, and improved tissue repair mechanisms bolstered by a more stable cellular environment and decreased oxidative stress (1,4).

Histological analysis also showed that the treated group had more fibroblast activity, quicker re-epithelialization, and a definite decrease in inflammatory cell infiltration as compared to the control. These results imply that the extract modifies the early inflammatory response, possibly by downregulating pro-inflammatory mediators like TNF-α and IL-1β, which are known to impede wound healing when continuously high (1).

Additionally, Masson's Trichrome staining showed that the treated group had better fiber structure and much more collagen deposition ($p < 0.05$). Crucially, this improvement represents both an increase in collagen amount and an improved structural arrangement, both of which are essential for minimizing excessive or disorganized scar formation and restoring tensile strength (20,21). Overall, our results show that Ficus carica extract has a multi-target effect on wound healing through better extracellular matrix remodeling, early inhibition of inflammation, stimulation of fibroblast activity, and improvement of collagen production. In line with other research emphasizing the function of plant-derived antioxidant chemicals in tissue regeneration, these effects taken together result in quicker and better wound repair when compared to the control group (22,23).

Table 1. Histomorphometric measurements of skin layers in control and *Ficus carica* groups (Mean ± SE).

Groups	Epidermis	Dermis	Hypodermis
Control 3	44.489 ± 3.554 b	386.177 ± 26.069 ab	249.899 ± 5.704 b
Ficus carica 3	78.908 ± 3.82 a	436.627 ± 24.793 a	296.37 ±6.4104 a
Control 7	84.869 ± 4.68 ab	319.95 ± 20.445 a	309.275 ± 9.34 a
Ficus carica 7	73.106 ± 2.492 b	333.752 ± 13.13 a	333.26 ± 18.66 a
Control 14	101.032± 5.246 b	278.132 ± 19.51 b	311.181 ± 32.38 a
Ficus carica 14	105.787 ± 7.233 b	369.772 ± 8.107 a	355.89 ± 20.105 a
Control 21	144.101 ± 3.268 b	341.907 ± 8.322 a	328.903 ± 0.935 a
Ficus carica 21	168.624	322.72	342.28

	± 6.257 a	± 7.477 a	± 11.656 a
Ficus carica 21	168.624 ± 6.257 a	322.72 ± 7.477 a	342.28 ± 11.656 a

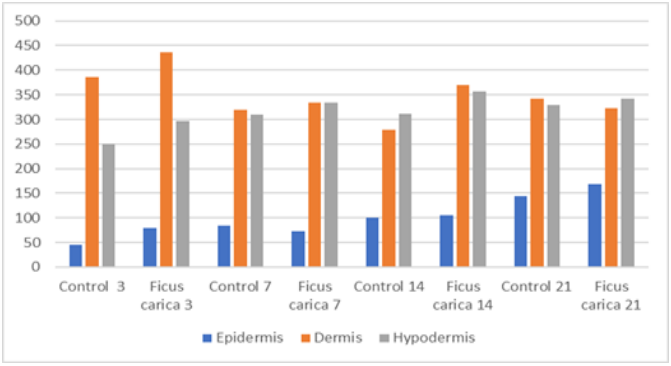


Figure 1. Effect of *Ficus carica* on Skin Layer Thickness During Wound Healing.

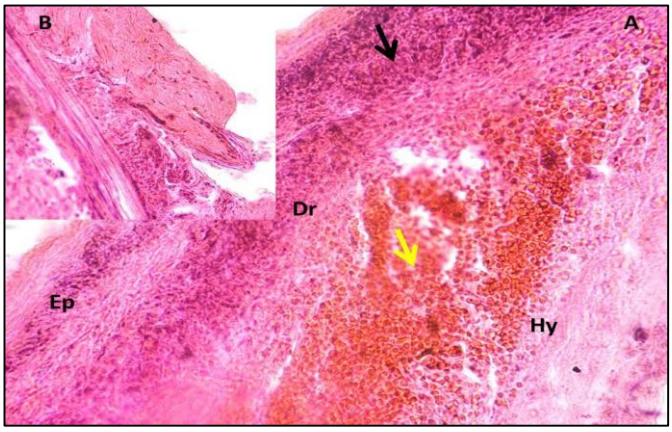


Figure 2. photography of skin (control group) reveals that the A Erosion of the epidermis (EP) with severe granulation tissue (black arrow).and high congestion in dermis (Dr) and extend in hypodermis layer (HY) yellow arrow). H&E stain.400x.

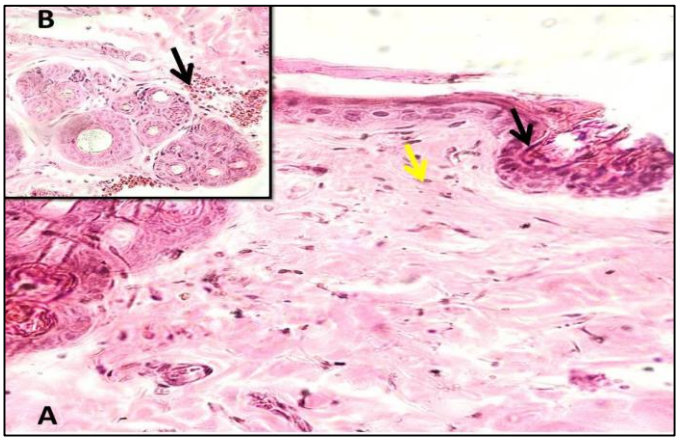


Figure 3. Photography of skin (3-day Fig group) reveal that the A- Re- epithelialization better than control group (black arrow). In dermis layer observed reduce granulation tissue (yellow arrow). B- Regeneration slightly of hair follicles and scant amount of hemorrhage H&E stain. 100x.

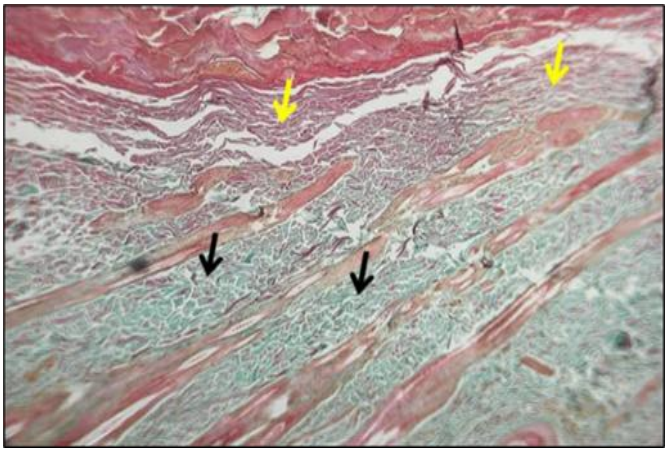


Figure 4. Photography of skin (control group) reveal that the scant of collagen fibers (green color) (black arrows) appears thin and fragmented. Masson trichrome. 100X

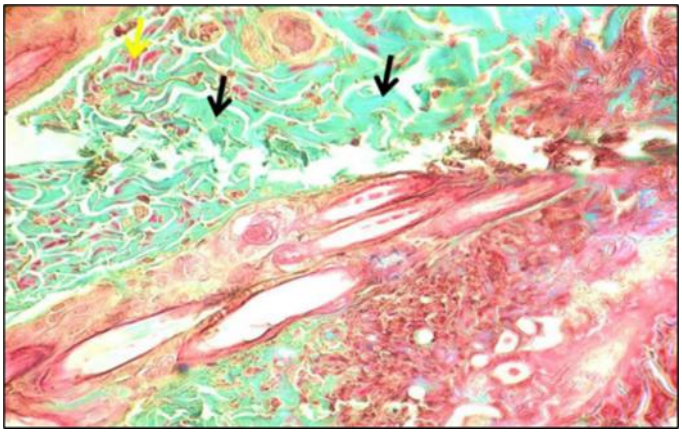


Figure 5. Photography of skin (Fig aurantium group) reveal that the thick bundles of collagen fibers around hair follicles (black arrows). Masson trichrome. 100X.

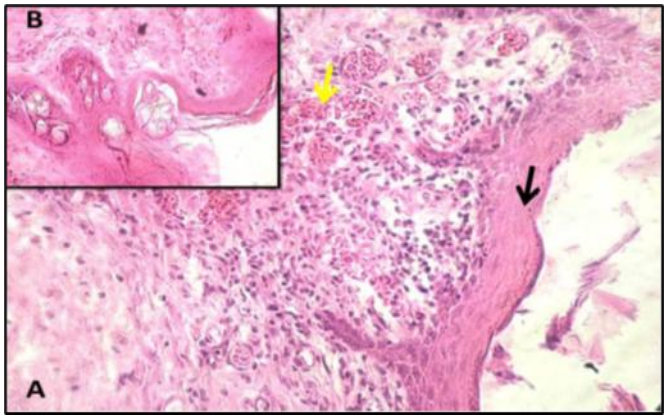
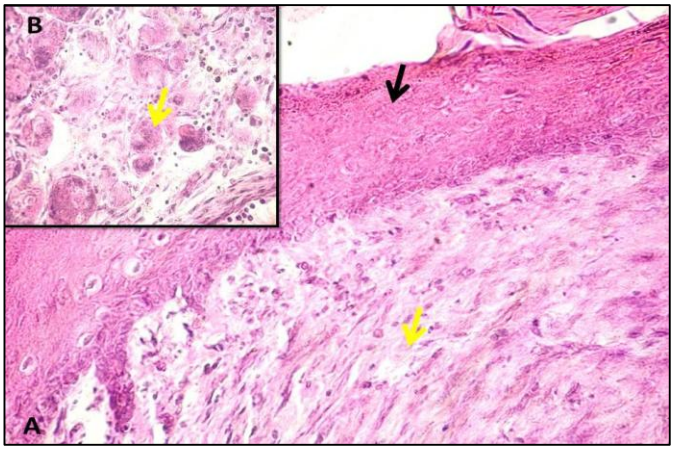


Figure 6. Photography of skin (control group) demonstrated a full re-epithelialization in epidermal layer and not observed any damage in epidermal layer (black arrow) while, in papillary dermis present amount of infiltration inflammatory cells with full enhanced of angiogenesis (yellow arrow). B- In dermis show regeneration of hair follicles. H&E stain.400X.



.**Figure 7.** Photography of skin (Fig group) demonstrated completely re-epithelialization and thicker better than control group (black arrows). B- The reticular dermal layer had density regeneration of hair follicles. H&E stain. 400X.

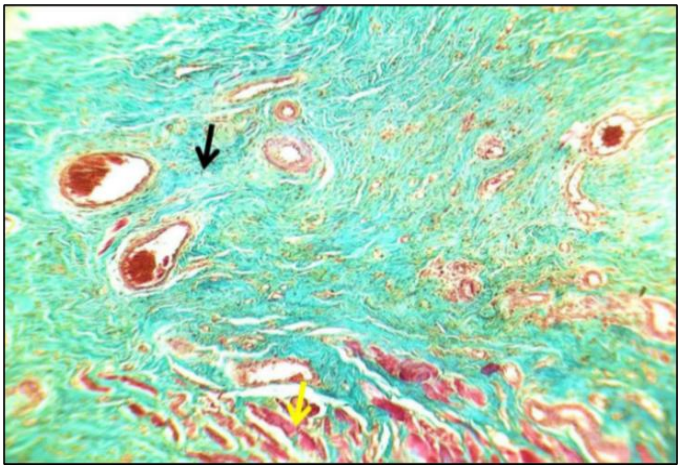


Figure 8. Photography of skin (control group) showed dense, thin, scattered, -stained collagen fibers are dispersed in-consistently throughout the dermis, indicating substantial but irregular accumulation of collagen. The collagen bundles were not oriented correctly. Masson trichrome. 400X.

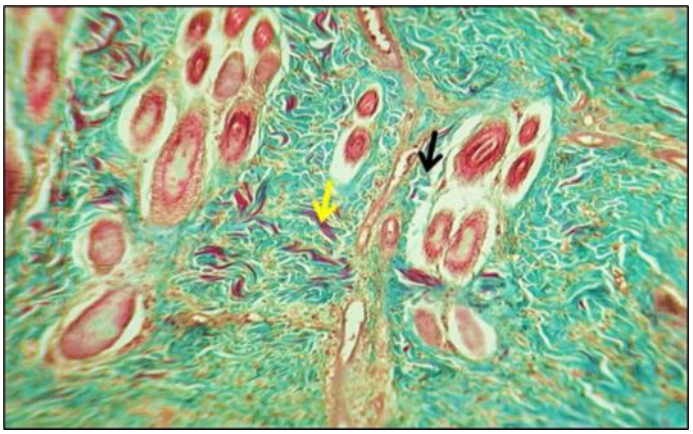


Figure 9. Photography of skin (Fig group) showed the collagen fibers greater than control group and these bundles scattered in a large amount around hair follicles and sweat glands (black arrows) with scant amount of muscles fibers. trichrome.400X.

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