ХІРУРГІЯ ТА АНЕСТЕЗІОЛОГІЯ

UDC 616-089.844:615.451.1:582.677.2

Autologous skin transplantation using water extract of bay leaves (*Laurus nobilis* L.)

Albozachri J.M.K., Khudaier A.M.

College of Veterinary Medicine, University of Kerbala, Karbala, Iraq

Альбозахрі Дж.М.К., Худаєр А.М. Аутологічна трансплантація шкіри з використанням водного екстракту лаврового листя (*Laurus nobilis* L.). Науковий вісник ветеринарної медицини, 2025. № 2. С. 145–150.

Albozachri J.M.K., Khudaier A.M. Autologous skin transplantation using water extract of bay leaves (*Laurus nobilis* L.). Nauk. vìsn. vet. med., 2025. № 2. PP. 145–150.

Рукопис отримано: 09.09.2025 р.
Прийнято: 23.09.2025 р.
Затверджено до друку: 27.11.2025 р.

Doi: 10.33245/2310-4902-2025-200-2-145-150

Assessing the impact of aqueous bay leaf (*Laurus nobilis*) extract on the histological development of skin graft healing in rabbits was the aim of this investigation. Group A (control) and Group B (treatment with bay leaf extract) were randomly assigned to sixteen clinically healthy adult rabbits of both sexes, weighing 1.25–2 kg. All animals underwent sterile surgical creation of full-thickness skin wounds (4 cm^2) bilaterally on the abdomen, with partial skin grafting at each site. Skin tissue biopsies were collected from both groups on days 3, 7, 14, and 21 for histological analysis. On day 3, Group B exhibited reduced dermal inflammation and edema compared to Group A. By day 7, the treated wounds showed enhanced collagen organization, hair follicle dilatation, and moderate vascular changes, while control wounds displayed pronounced inflammation. On day 14, treated wounds demonstrated extensive dermal fibrosis and mononuclear cell infiltration with endothelial hyperplasia, whereas control wounds showed severe sebaceous gland proliferation and inflammatory exudate. At day 21, Group B wounds showed more mature fibrosis and glandular proliferation than group A, which was still with minimal connective tissue regeneration, topically applied bay leaf aqueous extract significantly can accelerate skin graft healing in rabbit via enhancing inflammation modulation and tissue regeneration too, may be a natural therapeutic agent for treatment of other wound in future.

Keywords: bay leaves, skin grafting, rabbit, anti-inflammatory.

Introduction. Skin transplantation remains a critical surgical procedure for managing conditions that result in extensive skin loss, such as deep burns, chronic ulcers, and severe traumatic injuries [1, 2]. Autologous skin transplantation, which involves grafting skin from a donor site to a recipient site on the same individual, is the gold standard as it circumvents the issue of allogeneic immune rejection [3–5]. However, the success of auto grafts is not guaranteed; it can be compromised by localized complications, primarily an exaggerated inflammatory response at the graft site [6, 7].

This dysregulated immune reaction is characterized by the overproduction of pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β), and increased oxidative stress. These factors can lead to graft damage, impaired integration, and poor cosmetic outcomes with excessive scarring [8–10]. Therefore, strategies to modulate this initial immune response are crucial for enhancing graft survival and promoting optimal regeneration.

Natural plant extracts with known anti-inflammatory and immunomodulatory properties offer a promising therapeutic avenue. The bay leaf (*Laurus nobilis L.*) water extract is rich in bioactive compounds such as polyphenols and sesquiterpene lactones, which have demonstrated potent anti-inflammatory and antioxidant effects in various models [11,12]. We hypothesize that the topical application of this extract following autologous skin transplantation will suppress key pro-inflammatory pathways, mitigate oxidative damage, and consequently create a more favorable microenvironment for graft acceptance and tissue repair.

The aim of this study was to evaluate histologically the effect of an aqueous bay leaf extract (*Laurus nobilis* L.) on healing of skin grafts in rabbits in terms of inflammation, collagen synthesis, and tissue repair

Materials and Methods.

Ethical Approval. This study was approved by the Research Ethics Committee of the College of Veterinary Medicine, University of Kerbala (Approval No. 1412 P.G., issued March 15, 2024). All experimental procedures were conducted in accordance with institutional animal care guidelines and international animal welfare standards.

Experimental Animals. In this investigation, sixteen adult rabbits of both sexes, weighing 1.25 to 2.0 kg, were in good clinical health. The animals were divided into two equal groups at random: Group B (therapy group): Wounds treated with an aqueous extract of *Laurus nobilis* L., while Group A (Control group) received no therapy.

Every animal was kept separately in stainless-steel cages at the University of Kerbala's College of Veterinary Medicine under standard laboratory settings, which included a 12-hour light/dark cycle, a temperature of 22 ± 2 °C, and a relative humidity of 50–60%. The animals were acclimated for a week before the experiment and were provided with unlimited access to food and water.

Preparation of Aqueous Extract of *Laurus nobilis* L. The aqueous extract of *Laurus nobilis* L. leaves was made using the procedure outlined by Duda-Chodak and Tarko (2007) [14]. To put it briefly, 250 g of fresh bay leaves were thoroughly cleaned under running water and allowed to air dry for five to seven days at room temperature. A laboratory mixer was then used to break the dry leaves into a coarse powder. Using an electric mixer, the powder was mixed with 50 milliliters of deionized water and let to remain at room temperature for a full day. After filtering the mixture through Whatman No. 1 filter paper, the filtrate was gathered and kept at 4 °C until it was needed.

Surgical Procedure and Wound Induction. All animals were anesthetized using a combination of Tramadol hydrochloride (10%, Ibn Hayyan Pharm., Syria) at 15 mg/kg body weight and Ketamine hydrochloride (10%, Alfasan, Holland) at 50 mg/kg body weight, administered intramuscularly [15]. Following aseptic preparation, each rabbit underwent the creation of two full-thickness skin wounds (4 cm^2) on both lateral sides of the abdomen using a sterile surgical blade [13]. One wound was left to heal by secondary intention, and the other was closed using autologous skin grafting.

In Group B, Following the autologous transplantation procedure, the grafted area was managed as follows. The wound was topically applied with aqueous bay leaf extract using a sterile cotton swab or syringe. Immediately after, the entire wound area was covered with a primary layer of sterile, non-adhesive vaseline gauze to prevent the dressing from adhering to the graft. This was followed by a secondary, absorbent layer made of sterile gauze pads. Finally, the dressing was secured in place with a cohesive bandage wrap to provide protection and mild pressure. The dressings were changed, and the extract was re-applied, every day for five days. Group A received no treatment.

Skin Biopsy Collection and Histological Evaluation. Skin biopsy samples were collected from all rabbits under general anesthesia at each time point (days 3, 7, 14, and 21). General anesthesia was induced using the same protocol as for the initial surgery, involving an intramuscular injection of a combination of Ketamine hydrochloride (10%, Alfasan, Holland) at 50 mg/kg body weight and Tramadol hydrochloride (10%, Ibn Hayyan Pharm., Syria) at 15 mg/kg body weight. No euthanasia was performed, as biopsies were taken from the wound margin without sacrificing the animals. After being removed from the incision site, tissue samples were promptly preserved in 10% neutral-buffered formalin. The samples were prepared for light microscopic analysis after 48 hours, embedded in paraffin, sectioned at a thickness of 5 μm by microtome (Lica, China) and stained with hematoxylin and eosin (H&E) [16]. Histological features were evaluated to assess inflammation, collagen deposition, neovascularization, and tissue regeneration by integrated microscope camera system (Biozek, Holland).

Results. In the first three days after surgery, all animals remained clinically healthy and exhibited normal feeding and behavior. No signs of systemic illness or infection were observed in either group. Wound sites appeared macroscopically clean, and aseptic conditions were confirmed throughout the experimental period.

As early as day three, the histological examination showed that wounds in Group A (control) displayed a mild inflammatory reaction with dermal infiltration of inflammatory cells and moderate edema in the interstitial space. In addition to this, there was pronounced follicular dilatation, some vascular congestion of the blood vessels supplying the skin, and early phases of sebaceous gland growth Type 1 arrows indicate marked changes (Fig. 1). On the other hand, Group B (received treatment with bay leaf extract) exhibited less inflammatory infiltration compared to previous assessments which is hallmark sign of an improved outcome with less dermal edema or an preserved inflammatory response.

In day seven group B wounds demonstrated significant improvements, including well-organized collagen fiber bundles, notable dilatation of hair follicles, moderate vascular congestion, and dense infiltration of inflammatory cells (Fig. 2). These features indicated a transition from the inflammatory to the proliferative phase. In comparison, Group A tissues showed marked sebaceous gland proliferation, vascular congestion with evidence of thrombosis, and moderate inflammatory exudates.

In day fourteen wounds in Group B showed extensive dermal fibrosis with abundant collagen band formation, dense mononuclear cell infiltration, and endothelial hyperplasia. In contrast, Group A displayed disorganized dermal architecture with pronounced myofiber proliferation, severe sebaceous gland hyperplasia, and abundant inflammatory exudates (Fig. 3).

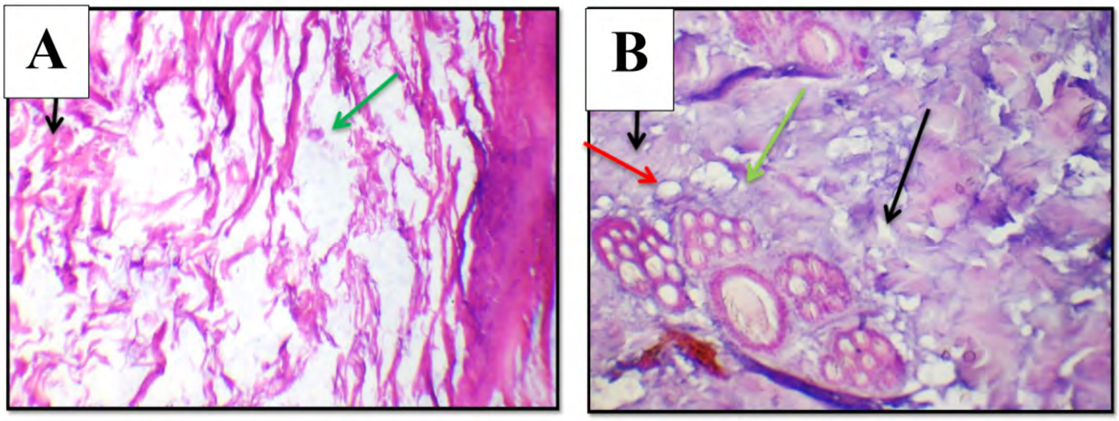

Fig. 1. **A cross-section of the skin histologically: (A) 3rd day revealed mild inflammatory dermal infiltration (black arrow), slight inflammatory edema (green arrow) (H and E, 10X). (B) 3rd day revealed significant proliferation of sebaceous glands (black arrow), sever hair follicle dilatation (green arrow) and mild vascular congestion (red arrow) (H and E, 10X).**

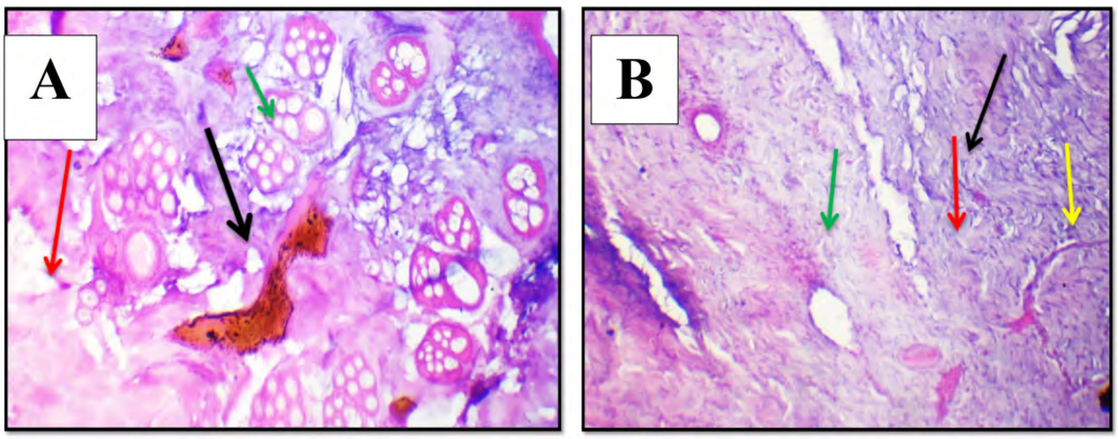

Fig. 2. **A cross-section of the skin histologically: (A) 7th day revealed significant congestion of blood vessels with significant thrombosis (black arrow), marked proliferation of sebaceous glands (green arrow) and moderate inflammatory exudation (red arrow) (H and E, 10X). (B) 7th day showed significant collagen bands proliferation (fibrous connective tissue) (black arrow), marked dilatation of hair follicles (green arrow), moderate vascular congestion (red arrow) and sever infiltration of inflammatory cells (yellow arrow) (H and E, 10X).**

In day twenty one group B samples exhibited mature fibrosis, dilated hair follicles, sebaceous gland proliferation, and minimal residual exudate, indicating near-complete tissue remodeling. Meanwhile, Group A presented only slight fibrous tissue proliferation with non-significant inflammatory exudation (Fig. 4).

The success of the graft is highly dependent on rapid revascularization. Delays in this process can result in partial graft necrosis. Furthermore, a pronounced inflammatory response at the graft site can impede integration and promote fibrosis [22].

Discussion. Wound healing is a highly coordinated process involving inflammation, cellular proliferation, extracellular matrix deposition, and tissue remodeling. The current study demonstrates that topical application of *Laurus nobilis* L. (bay leaf) aqueous extract significantly enhances the histological features associated with wound healing in rabbits undergoing skin graft procedures [17].

On day 3, reduced inflammatory infiltration in the treated group suggests that bay leaf extract is anti-inflammatory, presumably due to its bioactive compounds such as flavonoids and phenolic acids, which have been shown to inhibit protein denaturation and inflammatory cytokine production [17, 18].

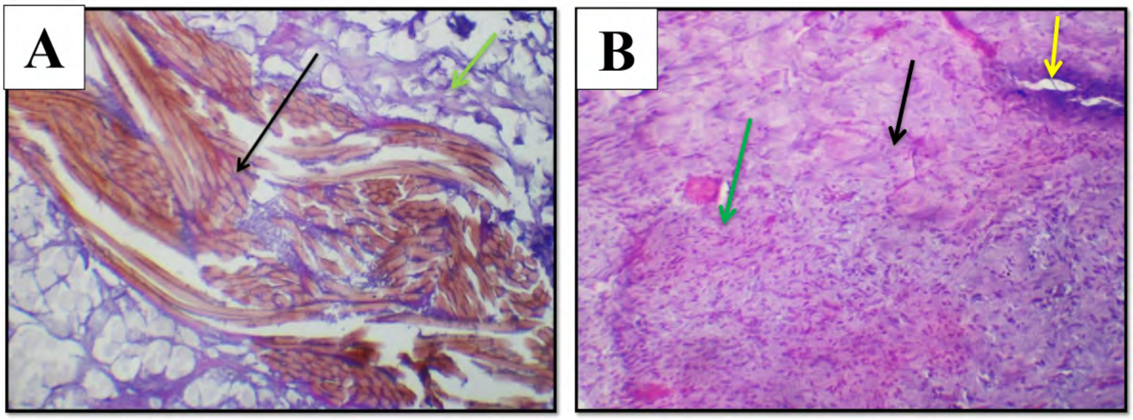

Fig. 3. **A cross-section of the skin histologically: (A) 14th day showed significant dermal myofibers proliferation (black arrow), inflammatory exudates (green arrow) (H and E, 10X). (B) 14th day reveals extensive dermal fibrosis (collagen bands proliferation) (black arrow), sever inflammatory cells infiltration mainly mononuclear cells (green arrow) with hyperplasia of blood vessels endothelia (yellow arrow) (H and E, 10X).**

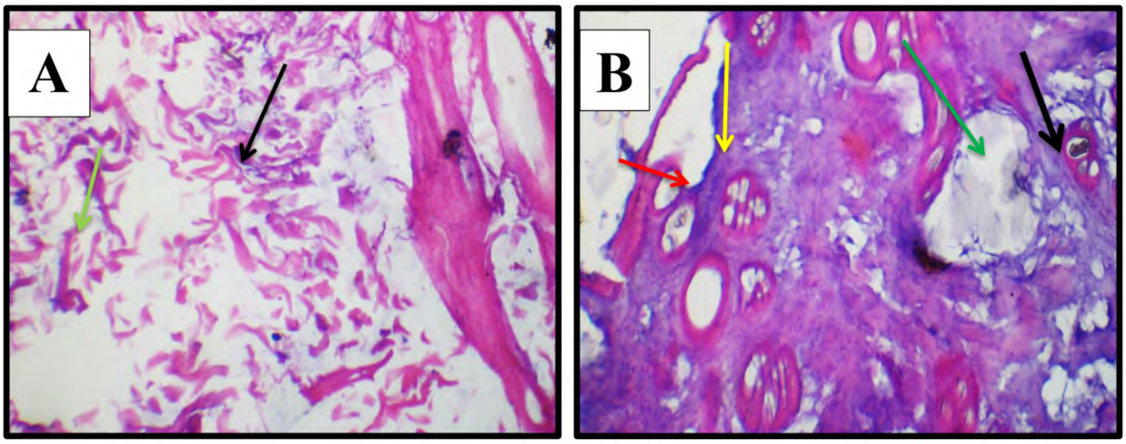

Fig. 4. **A cross-section of the skin histologically: (A) 21th day reveals slight fibrous connective tissue proliferation (black arrow), not remarkable inflammatory exudates (green arrow) (H and E, 10X). (B) 14th day reveals fibrosis (black arrow), inflammatory exudates (green arrow), hair follicles dilatation (red arrow), sebaceous glands proliferation (yellow arrow) (H and E, 10X).**

More collagen alignment and vascular activity were noted by day 7 in the treated group, which are important indicators of the proliferative phase. Correlation is direct between collagen fiber alignment and increased tensile strength, which is vital for sustaining long-term wound stability [19]. Lack of edema and tissue damage with diffuse inflammatory cell infiltration within treated wounds indicates controlled and productive inflammation for healing and tissue regeneration.

On day 14, the noted fibrosis, mononuclear infiltration, and endothelial hyperplasia in the treatment group are indicative of continued angiogenesis and matrix remodeling. This accords with prior findings that plant extracts can cause modulation of fibroblast activity and cause vascular growth [20].

By day 21, the treated group had more developed healing features, including mature fibrous tissue and reduced inflammation, whereas control wounds were at earlier stages of healing. This shows that bay leaf extract heals quickly as well as improves tissue quality [21].

The extract's effectiveness in promoting epithelial migration, angiogenesis, and collagen deposition suggests its potential use as a natural therapeutic agent in wound care. The positive impact observed in both normal and grafted tissues reinforces the utility of bay leaf extract in enhancing graft integration and minimizing complications such as inflammation and delayed healing.

The activation of regenerative processes in damaged skin during autologous transplantation is primarily mediated by a finely orchestrated immune response. Unlike allogeneic transplants, auto grafts circumvent major histocompatibility complex (MHC)-mediated rejection, allowing the immune system to focus on wound healing and tissue regeneration rather than graft destruction [22]. The key regenerative mechanisms include:

Attenuation of Pro-inflammatory Signaling: A critical shift from a pro-inflammatory to a pro-regenerative phase is essential. Early, controlled inflammation is necessary for debridement, but its prolonged state, characterized by elevated levels of cytokines such as TNF-α and IL-1β, can impede healing and damage the graft [23].

Promotion of Anti-inflammatory Pathways: The up regulation of anti-inflammatory cytokines, particularly IL-10 and TGF-β, plays a pivotal role. IL-10 dampens excessive inflammation and inhibits T-cell activation, while TGF-β is a potent stimulator of fibroblast proliferation and collagen deposition, which are crucial for reconstructing the extracellular matrix [24].

Activation of Pro-regenerative Immune Cells: Specific immune cell populations, such as M2 macrophages, are instrumental. These cells are activated in a supportive cytokine environment and contribute to tissue repair by secreting growth factors like VEGF (promoting angiogenesis) and PDGF (stimulating fibroblast migration) [25].

Conclusions. This study demonstrates that topical application of *Laurus nobilis L.* aqueous extract significantly enhances skin graft healing in rabbits through distinct histological improvements. The extract accelerated the inflammatory phase resolution, promoted organized collagen deposition, and facilitated advanced tissue remodeling compared to controls. These findings validate the extract's role as an effective natural wound-healing modulator. Future studies should focus on identifying the active compounds and evaluating clinical applications in wound management.

REFERENCES

1. Ayoub, N.A., Hashim, A.N., Hussein, S.A., Hegazi, N.M., Hassanein, H.M., Nawwar, M.A. (2013). Hepatoprotective effect of bay leaves crude extract on primary cultured rat hepatocytes. European Scientific Journal (ESJ), 9 (30), pp. 647–655.

2. Awada, F., Hamade, K., Kassir, M., Hammoud, Z., Mesnard, F., Rammal, H., Fliniaux, O. (2023). Laurus nobilis leaves and fruits: A review of metabolite composition and interest in human health. Applied Sciences. 13 (7), 4606 p. DOI:10.3390/app13074606.

3. Branski, L.K., Herndon, D.N., Barrow, R.E. (2007). A review of gene therapy in wound healing. International Journal of Burns and Trauma, 1 (1), pp. 1–12. DOI:10.1016/j.burns. 2008.03.009.

4. Sharangi, A.B., Guha, S. (2013). Wonders of leafy spices: Medicinal properties ensuring human health. Science International. 1 (9), pp. 312–317. DOI:10.17311/sciintl.2013.312.317.

5. Mishra, P.K., Tripathi, J., Gupta, S., Variyar, P.S. (2019). GC-MS olfactometric characterization of odor active compounds in cooked red kidney beans (Phaseolus vulgaris). Heliyon, 5 (9). DOI:10.1016/j.heliyon.2019.e02459

6. Landén, N.X., Li, D., Ståhle, M. (2016). Transition from inflammation to proliferation: a critical step during wound healing. Cellular and Molecular Life Sciences. 73 (20), pp. 3861–3885. DOI:10.1007/s00018-016-2268-0.

7. Baytop, T. (1985). Therapy with Medicinal Plants in Turkey (Past and Present). Istanbul: Nobel Tıp Kitabevleri, 194 p.

8. Aqili Khorasani, M. S. (1992). Collection of drugs (Materia medica). Engelab-e-Eslami Publishing and Educational Organization, pp. 624–630.

9. Barla, A., Topçu, G., Öksüz, S. (2007). Secondary metabolites from *Laurus nobilis L.* Natural Product Research. 21 (2), pp. 138–145.

10. Khodja, Y.K., Bachir-Bey, M., Ladjouzi, R., Katia, D., Khettal, B. (2021). *In vitro* antioxidant and antibacterial activities of phenolic and alkaloid extracts of *Laurus nobilis*. South Asian Journal of Experimental Biology, 11 (3). DOI:10.38150/sajeb.11(3).p345-354

11. DesJardins-Park, H.E., Gurtner, G.C., Wan, D.C., Longaker, M.T. (2022). From chronic wounds to scarring: the growing health care burden of under-and over-healing wounds. Advances in Wound Care. 11 (9), pp. 496–510. DOI:10.1089/wound.2021.0039

12. Fossum, T.W. (2018). Small Animal Surgery E-Book: Small Animal Surgery E-Book. Elsevier Health Sciences.

13. Bright, C.T. (1994). Fracture healing in the rabbit's fibula when subjected to various capacities couple electrical field. Journal of Orthopaedic Research, 3, pp. 331–340.

14. Mitic, V., Ilic, M., Dimitrijevic, M., Cvetkovic, J., Ciric, S., Jovanovic, V.S. (2016). Chemometric characterization of peach, nectarine and plum cultivars according to fruit phenolic content and antioxidant activity. Fruits, 71 (1), pp. 57–66. DOI:10.1051/fruits/2015042

15. Albozachri, J.M.K., Al-Tomah, H.M., Wali, O.N., Jameel, Y.J. (2019). A comparison study of nefopam-ketamine, tramadol-ketamine and xylazine-ketamine anesthesia in rabbit. Research Journal of Pharmacy and Technology, 12 (5), pp. 2439–2442. DOI:10.5958/0974-360X. 2019.00410.9

16. Grunberger, D., Banerjee, R., Eisinger, K., Oltz, E.M., Efros, L., Caldwell, M., Estevez, V., Nakanishi, K. (1988). Preferential cytotoxicity on tumor cells by caffeic acid phenethyl ester isolated from propolis. Experientia, 44 (3), pp. 230–232. DOI:10.1007/BF01941717

17. Almadani, Y.H., Vorstenbosch, J., Davison, P.G., Murphy, A.M. (2021). Wound healing: a comprehensive review. In Seminars in plastic surgery. Thieme Medical Publishers, Inc. Vol. 35, no. 03, pp. 141–144. DOI:10.1055/s-0041-1731791.

18. Irvin, T.T. (1995). Surgical wound healing in rats. Journal of Biological Chemistry, 290, pp. 14854–14860.

19. Gushiken, L.F.S., Beserra, F.P., Bastos, J.K., Jackson, C.J., Pellizzon, C.H. (2021). Cutaneous wound healing: An update from physiopathology to current therapies. Life, 11 (7), 665 p. DOI:10.3390/life11070665.

20. Solmaz, H., Dervisoglu, S., Gulsoy, M., Ulgen, Y. (2016). Laser biostimulation of wound healing: bioimpedance measurements support histology. Lasers in medical science, 31 (8), pp. 1547–1554. DOI:10.1007/s10103-016-2013-9

21. Loots, M.A.M., Kenter, S.B., Au, F.L. (2002). Fibroblasts derived from chronic diabetic ulcers differ in their response to stimulation with EGF, IGF-I, bFGF, and PDGF-AB compared to controls. European Journal of Cell Biology, 81 (3), pp. 153–160. DOI:10.1078/0171-9335-00228.

22. Gurtner, G.C., Werner, S., Barrandon, Y., Longaker, M.T. (2008). Wound repair and regeneration. Nature, 453 (7193), pp. 314–321. DOI:10.1038/nature07039.

23. Eming, S.A., Martin, P., Tomic-Canic, M. (2014). Wound repair and regeneration: mechanisms, signaling, and translation. Science Translational Medicine, 6 (265). DOI:10.1126/scitranslmed.3009337.

24. Barrientos, S., Stojadinovic, O., Golinko, M.S., Brem, H., Tomic-Canic, M. (2008). Growth factors and cytokines in wound healing. Wound Repair and Regeneration, 16 (5), pp. 585–601. DOI:10.1111/j.1524-475X.2008.00410.x.

25. Murray, P.J., Wynn, T.A. (2011). Protective and pathogenic functions of macrophage subsets. Nature Reviews Immunology, 11 (11), pp. 723–737. DOI:10.1038/nri3073.

Аутологічна трансплантація шкіри з використанням водного екстракту лаврового листя (*Laurus nobilis L.*)

Альбозахрі Дж.М.К., Худаєр А.М.

Метою цього дослідження була оцінка впливу водного екстракту лаврового листа (*Laurus nobilis*) на гістологічний розвиток загоєння шкірного трансплантата у кроликів. Група А (контрольна) та група В (лікування екстрактом лаврового листа) були випадковим чином розподілені по шістнадцять клінічно здорових дорослих кроликів обох статей вагою 1,25–2 кг. Усім тваринам було проведено стерильне хірургічне створення повнотовстої шкірної рани (4 см²) двосторонньо на животі з частковою шкірною пластикою в кожній ділянці. Біопсії тканини шкіри були зібрані в обох групах на 3, 7, 14 та 21-шу добу для гістологічного аналізу. На 3-тю добу у групі В спостерігалося зменшення запалення та набряку шкіри порівняно з групою А. До 7-ї доби оброблені рани демонстрували покращену організацію колагену, розширення волосяних фолікулів та помірні судинні зміни, тимчасом у контрольних ранах спостерігалося виражене запалення. На 14-ту добу у оброблених ранах спостерігався значний дермальний фіброз та інфільтрація мононуклеарними клітинами з ендотеліальною гіперплазією, тимчасом у контрольних ранах спостерігалася виражена проліферація сальних залоз та запальний ексудат. На 21-шу добу рани групи В демонстрували більш зрілий фіброз та залозисту проліферацію, ніж група А, яка все ще мала мінімальну регенерацію сполучної тканини. Місцево застосовуваний водний екстракт лаврового листа може значно пришвидшити загоєння шкірного трансплантата у кроликів завдяки посиленню модуляції запалення та регенерації тканин, що також може бути природним терапевтичним засобом для лікування інших ран у майбутньому.

Ключові слова: лавровий лист, шкірна пластика, кролик, протизапальний засіб.

ORCID iD:
Albozachri J.M.K. https://orcid.org/0000-0002-7115-3528