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Morphological and Ultrastructural Evaluation of Propolis in Rabbit Oral Cavity Wound Healing

Article Info.

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Abstract

Propolis, a natural resinous substance produced by honeybees, possesses anti-inflammatory, antioxidant, antimicrobial, and tissue-regenerative properties. This study evaluated the effects of topical propolis gel on hard palate wound healing in rabbits. Forty-eight adult male rabbits were divided into control groups (C3, C7, C14, C21) treated with base gel and treatment groups (T3, T7, T14, T21) treated with propolis gel. Standardized 10 × 3 mm palatal incisions were created, and healing was assessed morphologically and ultrastructurally. Wound contraction was significantly accelerated in treated groups from day 7 onward, with final diameters of ~2 mm compared to ~5 mm in controls ($p \leq 0.0001$). Histological and scanning electron microscopic examinations revealed organized collagen fibers, abundant fibroblasts, and complete epithelial closure in treated wounds, whereas control wounds exhibited delayed remodeling and persistent inflammation. In conclusion, the topical propolis gel significantly enhanced oral wound healing, confirming its potential as a natural therapeutic agent.

Keywords: Propolis, Collagen, Morphology, Ultrastructure, Wound healing

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Introduction

Oral cavity injuries are common in both clinical and experimental settings and often present challenges for effective and rapid tissue repair. Wound healing in the oral mucosa involves a complex interplay of cellular proliferation, collagen deposition, and tissue remodeling. Delayed or impaired healing can lead to persistent inflammation, infection, and compromised oral function (1).

Propolis, a resinous substance produced by honeybees, has gained attention as a natural therapeutic agent due to its anti-inflammatory, antioxidant, antimicrobial, and tissue-regenerative properties (2). Several studies have demonstrated that propolis can accelerate wound closure, promote fibroblast proliferation, and enhance collagen organization in skin and mucosal injuries (3). Its biocompatibility and minimal side effects make it an attractive alternative to conventional synthetic agents in wound management (4).

Despite increasing evidence of its efficacy, limited studies have comprehensively evaluated the morphological and ultrastructural changes induced by propolis during oral wound healing. Scanning electron microscopy (SEM) offers high-resolution visualization of collagen fiber arrangement, fibroblast activity, and tissue remodeling, providing critical insights into the regenerative processes at the cellular and subcellular levels (5).

The present study aimed to investigate the effects of topical propolis gel on hard palate injuries in rabbits, with a particular focus on wound contraction, tissue morphology, and ultrastructural organization, to better understand its potential as a natural agent for enhancing oral tissue repair.

Materials and Methods

This experiment was carried out under the approval and supervision of the Animal Ethics Committee, College of Veterinary Medicine, University of Basrah. All procedures complied with the international standards for the use and care of laboratory animals (6). Forty-eight healthy adult male Iraqi rabbits (*Oryctolagus cuniculus*), with an average body weight ranging from 1.8 to 2.2 kg, were used. Animals were housed individually in stainless-steel cages within a controlled environment maintained at 22 ± 2 °C, with 50–60% relative humidity and a 12-hour light/dark cycle. Standard commercial pellets and fresh water were made available *ad libitum*. Before experimentation, all rabbits were acclimatized for one week to minimize stress (6). The rabbits were randomly divided into eight experimental groups (six animals per group). Four

groups served as controls (C3, C7, C14, and C21), where wounds were treated daily with a gel base free of propolis. The remaining four groups represented the treatment sets (T3, T7, T14, and T21), which received topical applications of propolis gel once per day.

Group codes (3, 7, 14, 21) indicated the time points, in days post-incision, when animals were euthanized for analysis. The random allocation of animals into groups followed recommended principles for experimental design (7).

General anesthesia was induced by intramuscular administration of ketamine hydrochloride (35 mg/kg) combined with xylazine (5 mg/kg). After achieving an adequate anesthetic depth, each rabbit underwent a standardized longitudinal incision (10 × 3 mm) along the midline of the hard palate, performed under aseptic conditions using a sterile scalpel. Following wound creation, the appropriate gel (either propolis or placebo) was applied topically according to the group assignment, and treatment continued daily until the scheduled sacrifice (8). The progression of healing was monitored by measuring wound diameters at each evaluation point (days 3, 7, 14, and 21). These measurements were used to calculate wound contraction and to assess morphological changes in the healing process (9).

For ultrastructural analysis, tissue samples were excised at the defined time intervals. Specimens were fixed in 2.5% glutaraldehyde, dehydrated in ascending ethanol concentrations, and then coated with a thin layer of gold using a sputter coater. Prepared samples were examined under a scanning electron microscope (SEM) to evaluate the organization of collagen fibers, density of fibroblasts, extracellular matrix deposition, and wound closure patterns (10).

All data were expressed as mean ± standard deviation (SD). The results were analyzed using one-way analysis of variance (ANOVA) to determine significant differences among groups, followed by Tukey's post hoc test for multiple comparisons. Statistical analysis was performed using SPSS software, version 27.0 (IBM Corp., Armonk, NY, USA). A *p*-value less than 0.05 was considered statistically significant (11).

Results

Morphometric assessment: The macroscopic evaluation of wound contraction revealed progressive healing across all groups, with distinct differences between the control and treatment groups (Figures 1 and 2) and (Table 1).

On day 3 post-incision, no statistically significant difference was observed between the control (C3: 9.00 ± 0.34 mm) and treatment (T3: 8.00 ± 0.34 mm) groups ($p \geq 0.01$). By day 7, a highly significant reduction in wound size was recorded in the treatment group (T7: 6.00 ± 0.34 mm) compared with the control group (C7: 8.00 ± 0.34 mm; $p \leq 0.0001$). A similar pattern was observed on day 14, where wound contraction was significantly enhanced in the treatment group (T14: 4.00 ± 0.34 mm) compared with the control group (C14: 7.00 ± 0.34 mm; $p \leq 0.0001$). The most pronounced difference was observed on day 21, as the treatment group (T21: 2.00 ± 0.34 mm) showed significantly greater wound closure compared with the control (C21: 5.00 ± 0.34 mm; $p \leq 0.0001$). Overall, the results indicate that propolis gel treatment markedly accelerated wound contraction compared with the control, with significant improvements evident from day 7 onward.

Table (1): Mean wound incision sizes (mm ± SD) in control and treatment groups at different evaluation times

Evaluation Day	Control Group (C)	Treatment Group (T)	p-value	Significance
Day 3 (C3 vs. T3)	9.00 ± 0.34	8.00 ± 0.34	≥0.01	ns (non-significant)
Day 7 (C7 vs. T7)	8.00 ± 0.34	6.00 ± 0.34	≤0.0001	*** (highly significant)
Day 14 (C14 vs. T14)	7.00 ± 0.34	4.00 ± 0.34	≤0.0001	*** (highly significant)
Day 21 (C21 vs. T21)	5.00 ± 0.34	2.00 ± 0.34	≤0.0001	*** (highly significant)

Note: ns = not significant; *** = highly significant.

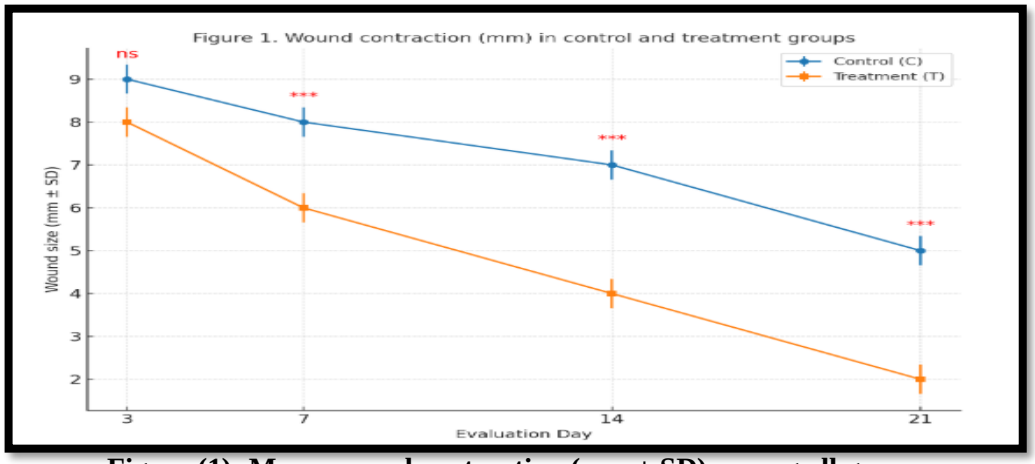

Figure (1): Mean wound contraction (mm ± SD) among all groups.

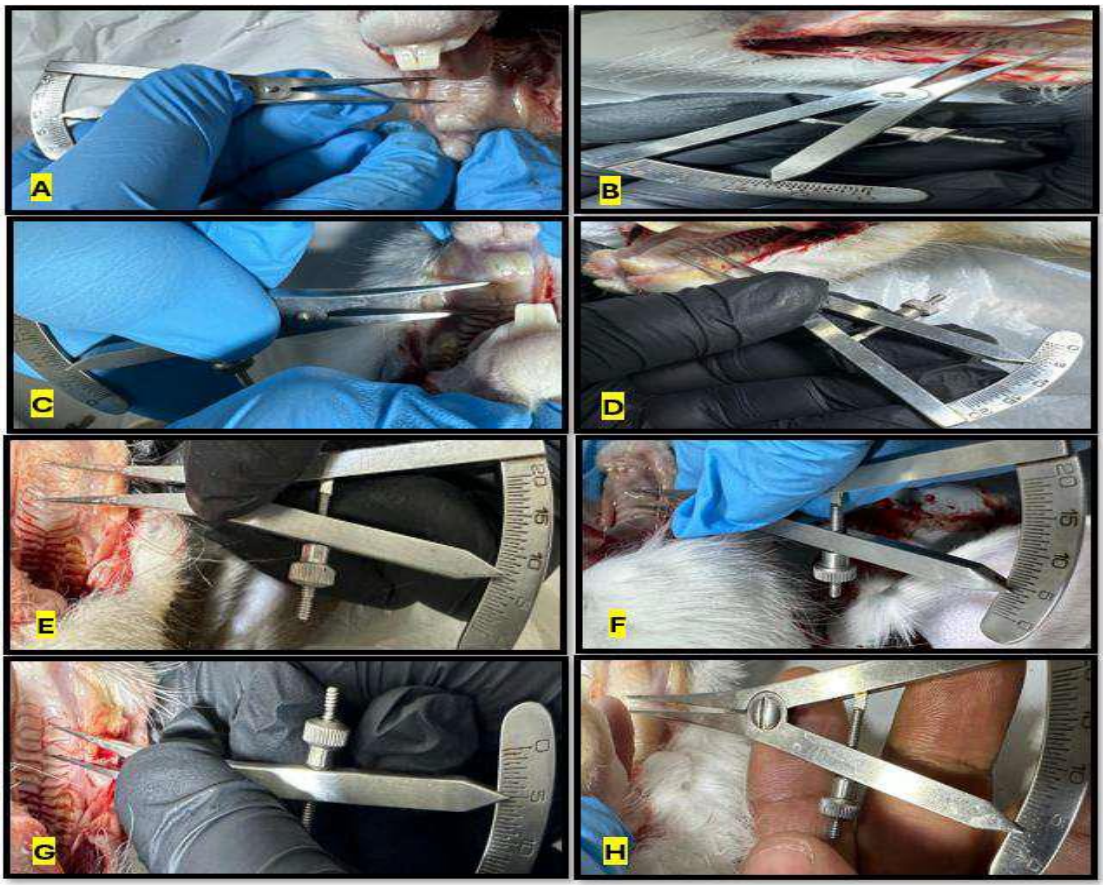

Figure 2: Morphometric measurements of hard palate wound healing showed A. wound healing of C3 sized 9 mm in diameter; B. wound healing of T3 sized 8 mm in diameter; C. wound healing of C7 sized 8 mm in diameter; D. wound healing of T7 sized 6 mm in diameter; E. wound healing of C14 sized 7 mm in diameter; F. wound healing of T14 sized 4 mm in diameter; G. wound healing of C21 sized 5 mm in diameter; H. wound healing of T21 sized 2 mm in diameter.

Ultrastructural Analysis of Wound Healing

Scanning electron microscopy (SEM) revealed pronounced differences in wound healing between control and treated groups, as shown in (Table 2). At day 3 (C3), control wounds exhibited open margins with sparse collagen fibers and minimal inflammatory cells, reflecting a nascent inflammatory phase (Figure 3). In treated wounds (T3), dense macrophage and neutrophil infiltration accompanied early collagen deposition and emerging fibroblasts, indicating accelerated inflammatory progression (Figure 4).

By day 7 (C7), control wounds displayed limited macrophage presence and scattered collagen fibers, consistent with delayed proliferative onset (Figure 5). In contrast, treated wounds (T7)

demonstrated substantial macrophage infiltration, a dense collagen fiber network bridging the wound margins, and partial closure, indicative of active proliferation (Figure 6).

At day 14 (C14), control wounds maintained irregular collagen meshwork with incomplete closure and mild macrophage deposition, signaling postponed proliferation (Figure 7). Treated wounds (T14) exhibited uniform dense collagen deposition, complete wound closure, and notable fibroblast infiltration, highlighting early maturation and effective tissue remodeling (Figure 8).

By day 21 (C21), control wounds retained dense collagen yet partial closure (Figure 9), whereas treated wounds (T21) achieved full closure, organized collagen networks, and active re-epithelialization, demonstrating complete maturation (Figure 10).

Table (2): Summarized Scanning Electron Microscopy Findings

Group / Time	Wound Status	Collagen Fibers	Inflammatory Cells	Fibroblast Presence	Healing Phase
C3	Open	Few scattered fibers	Sparse neutrophils/macrophages	Minimal	Early inflammation
T3	Partially closed	Early meshwork	Dense neutrophils/macrophages	Early	Mature inflammation
C7	Open	Limited fibers	Minimal macrophages	Few	Early proliferation
T7	Partially closed	Dense meshwork	Heavy macrophages	Present	Active proliferation
C14	Partially open	Irregular dense	Mild macrophages	Present	Continued proliferation
T14	Closed	Dense uniform	Moderate macrophages	Present	Early maturation
C21	Partially closed	Dense meshwork	Present	Present	Delayed maturation
T21	Fully closed	Dense organized	Present	Present	Active maturation

Overall, SEM findings demonstrate that treated wounds progressed more rapidly through inflammatory, proliferative, and maturation phases compared to controls, achieving earlier and more organized tissue repair.

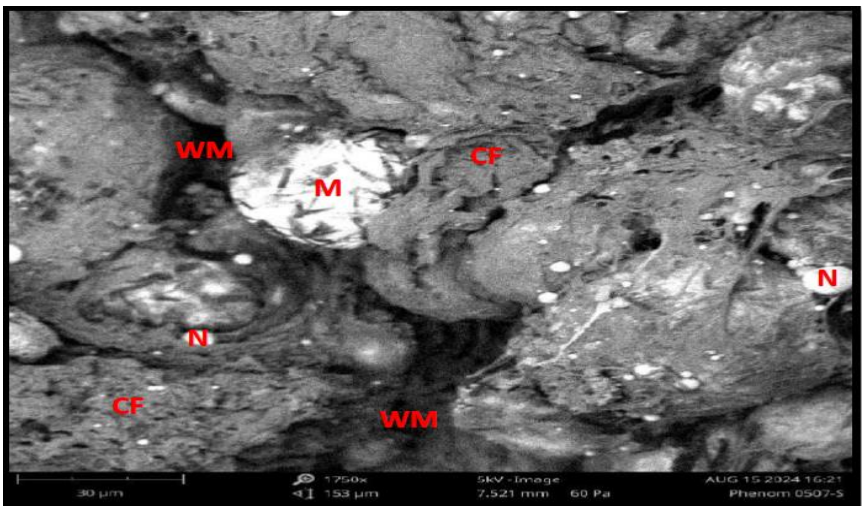

Figure 3: SEM image of the C3 group showing an open wound margin (WM) with scattered collagen fibers (CF) and few neutrophils (N) and macrophages (M), indicating early inflammation. (SEM ×1750; uranyl acetate stain; scale bar = 30 µm)

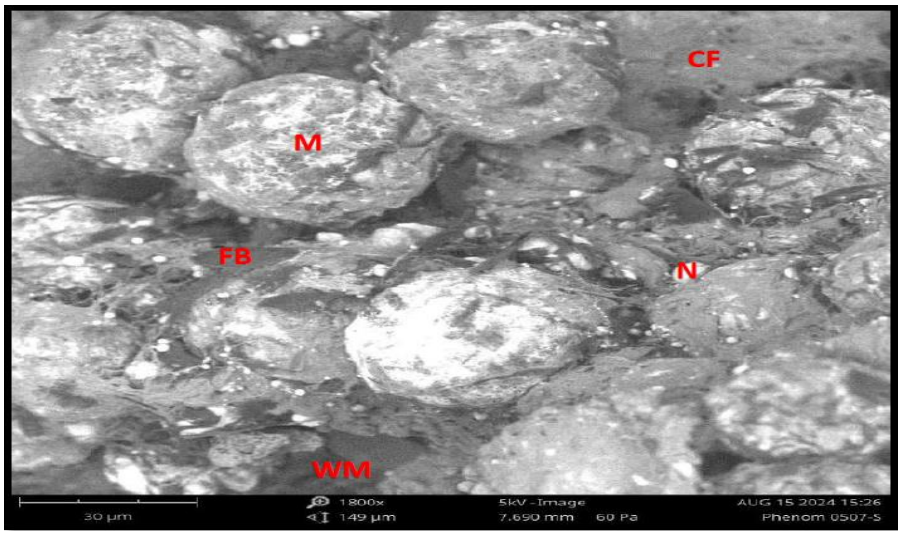

Figure 4: SEM image of the T3 group showing dense infiltration of macrophages (M) and neutrophils (N), early collagen fiber (CF) deposition, and initial fibroblasts (FB) at the wound margin (WM), indicating mature inflammation. (SEM ×1800; uranyl acetate stain; scale bar = 30 µm).

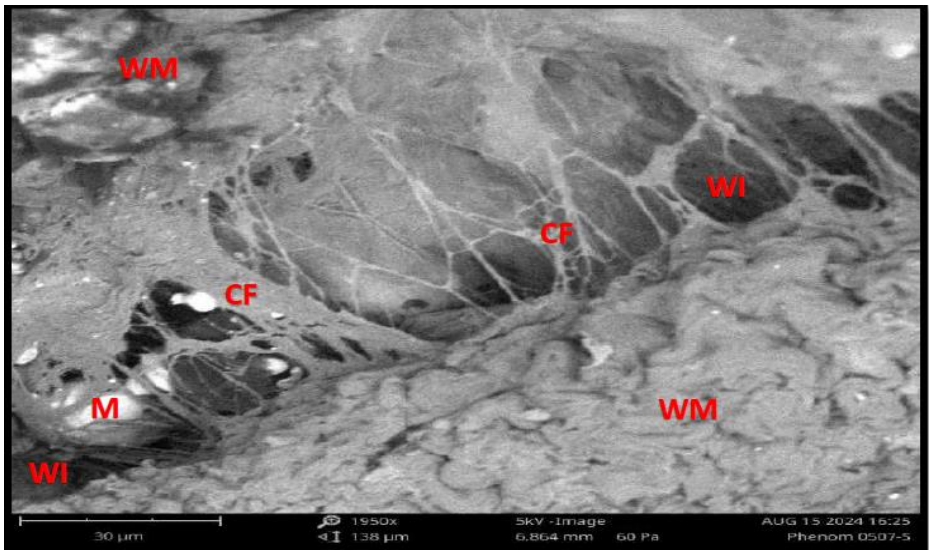

Figure 5: SEM image of the C7 group showing few macrophages (M) and limited collagen fibers (CF) at the wound margins (WM), indicating the early proliferative stage while the wound is still open. (SEM ×1950; uranyl acetate stain; scale bar = 30 µm).

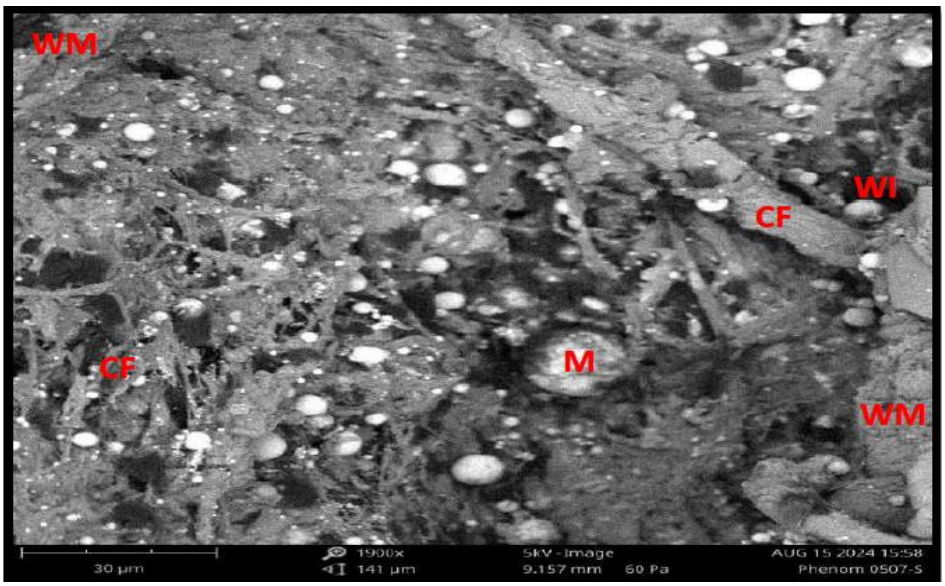

Figure 6: SEM image of the T7 group showing dense macrophage (M) infiltration and abundant collagen fibers (CF) at the wound margins (WM), resulting in wound closure and indicating active proliferation. (SEM ×1900; uranyl acetate stain; scale bar = 30 µm).

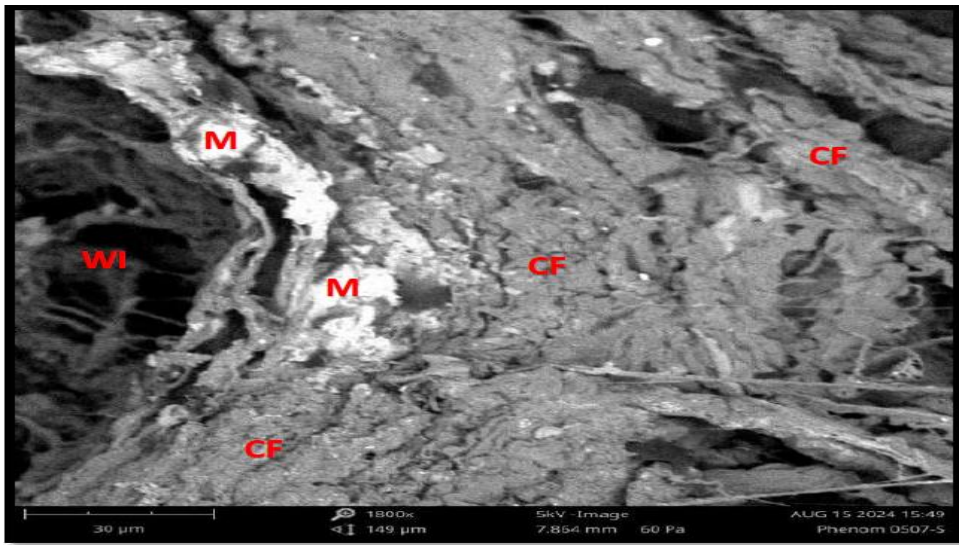

Figure 7: SEM image of the C14 group showing an irregular dense collagen fiber (CF) network at the wound incision (WI) with mild macrophage (M) presence. The wound is partially open, indicating continued proliferation and delayed maturation. (SEM ×1800; uranyl acetate stain; scale bar = 30 µm).

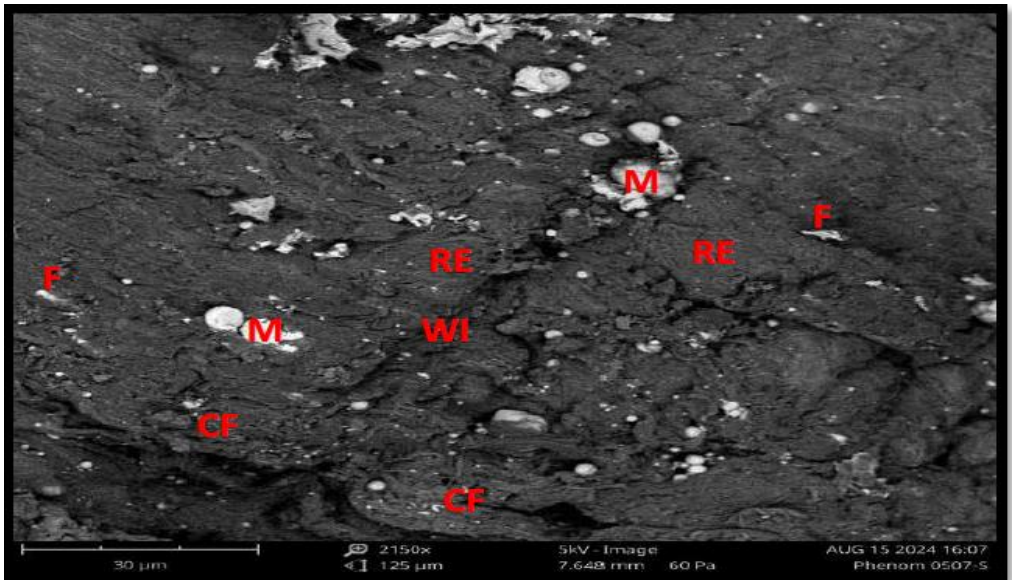

Figure 8: SEM image of the T14 group showing dense, regular collagen fibers (CF) at the wound margins (WM) with complete wound closure. Macrophages (M) and fibroblasts (F) are present, indicating re-epithelialization (RE) and early active maturation. (SEM ×2150; uranyl acetate stain; scale bar = 30 µm).

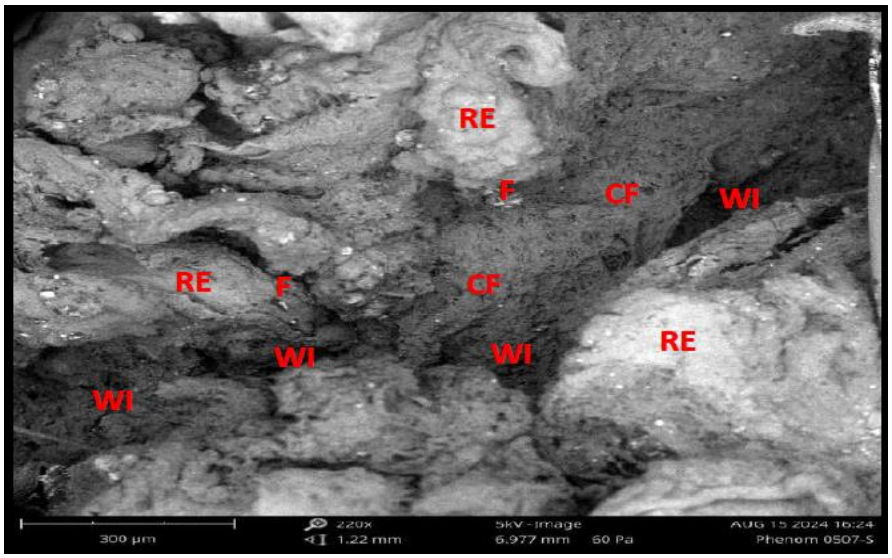

Figure 9: SEM image of the C21 group showing a dense collagen fiber (CF) network at the wound incision (WI) with fibroblast (F) infiltration, indicating early re-epithelialization (RE). The wound remains partially open, reflecting delayed maturation. (SEM ×220; uranyl acetate stain; scale bar = 300 µm).

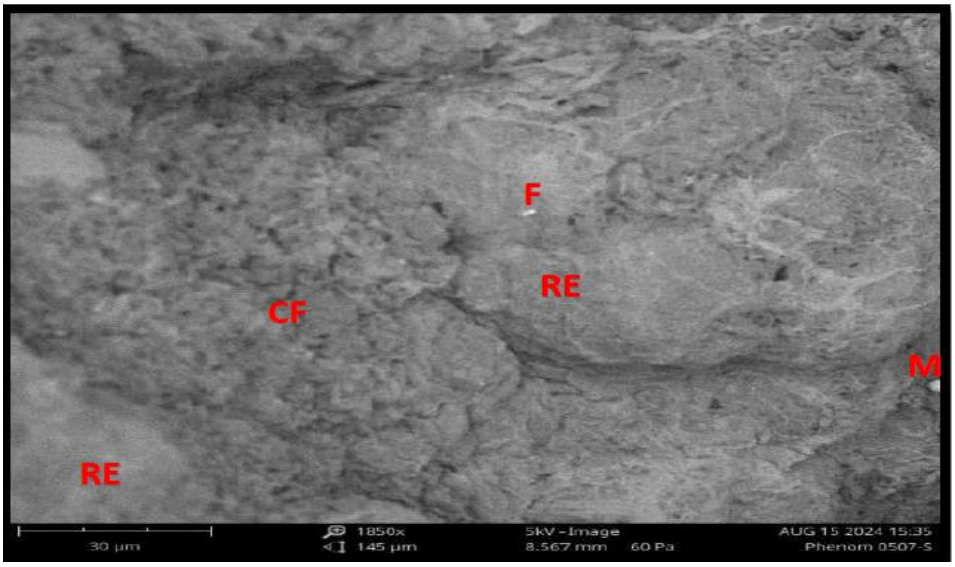

Figure 10: SEM image of the T21 group showing dense collagen fibers (CF) at the wound incision with full closure of wound margins (WM). Fibroblasts (F) and macrophages (M) are present, indicating complete re-epithelialization (RE) and active maturation. (SEM ×1850; uranyl acetate stain; scale bar = 30 µm).

Discussion

The present study demonstrates that propolis significantly accelerates the healing of hard palate wounds. Morphometric analysis revealed a marked reduction in wound margins in the propolis-treated group compared to controls, indicating faster tissue regeneration. This effect is likely to result from propolis strengthening the wound matrix and acting as a mucoadhesive agent, which protects the wound from contamination and promotes efficient closure (12). These findings align with previous studies highlighting the benefits of propolis in oral medicine, including its use in managing aphthous ulcers (13). Ultrastructural observations using SEM supported these morphometric findings. Early-stage control wounds (C3) exhibited open wound margins with scattered collagen fibers and limited neutrophil and macrophage infiltration, reflecting the initial inflammatory phase. In contrast, treated wounds (T3) showed dense macrophage infiltration, early collagen fiber deposition, and initial fibroblast presence, suggesting accelerated inflammation and initiation of tissue repair.

By day 7, control wounds (C7) demonstrated minimal collagen deposition and macrophage presence, whereas treated wounds (T7) displayed dense collagen networks and extensive macrophage coverage, indicating active proliferation and partial closure.

At later stages, control wounds (C14 and C21) showed irregular collagen deposition, limited fibroblast infiltration, and incomplete closure, suggesting delayed maturation. In contrast, treated wounds (T14 and T21) exhibited dense, well-organized collagen fibers, robust fibroblast and macrophage infiltration, and complete wound closure, reflecting full re-epithelialization and active remodeling. These findings indicate that propolis accelerates wound remodeling, enabling complete tissue regeneration within 21 days post-injury.

The accelerated healing in treated wounds may be attributed to bioactive compounds in propolis, such as p-coumaric acid and artepillin C, which enhance wound hydration and inhibit prostaglandin E2 and nitric oxide, preventing crust formation and reducing inflammatory exudate (14). Furthermore, metal ions like zinc and iron facilitate collagen synthesis, while flavonoids and phenolic acids reduce inflammation through modulation of the lipoxygenase pathway (Balata et al., 2018). Caffeic acid phenethyl ester provides cytoprotective and antioxidant effects, mitigating oxidative stress in injured tissues (15).

Functionally, propolis minimized local inflammation, prevented tissue necrosis, and promoted hard tissue formation, as evidenced by organized collagen deposition and complete re-epithelialization. Its regenerative and anti-inflammatory properties have been shown to induce full hard tissue barrier formation in pulpotomy procedures, confirming its safety and efficacy in oral wound management (16). Collectively, these findings demonstrate that propolis accelerates wound closure, enhances fibroblast and keratinocyte activity, and improves tissue organization, supporting its potential as a safe and effective therapeutic agent for oral wound healing.

Conclusion

The propolis significantly accelerates hard palate wound healing by promoting collagen deposition, enhancing fibroblast and keratinocyte activity, reducing inflammation, and improving tissue organization. Treated wounds exhibited faster closure, complete re-epithelialization, and advanced remodeling compared to controls. These findings support the potential of propolis as a safe and effective natural therapeutic agent for oral wound management.

Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical Clearance

This work is approved by The Research Ethical Committee

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التقييم الشكلي والفوق مجهري للعكبر في التئام جروح التجويف الفمي في الارانب

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الخلاصة

يُعتبر العكبر مادة راتنجية طبيعية ينتجها النحل، معروفًا بخصائصه المضادة للالتهابات، ومضادات الأكسدة، والمضادة للميكروبات، وقدرته على تعزيز تجدد الأنسجة. ومع ذلك، لا تزال إمكانياته في تحسين التئام جروح تجويف الفم غير مستكشفة بشكل كامل. هدفت هذه الدراسة إلى دراسة تأثيرات هلام العكبر الموضعي على مورفولوجيا وتركيب الجروح في الحنك الصلب لدى الأرانب. قُسم ثمانية وأربعون أرنبًا ذكرًا بالغًا من نوع *Oryctolagus cuniculus* بشكل عشوائي إلى ثماني مجموعات: أربع مجموعات سيطرة C3 ، C7، C14، C21 حيث عولجت يوميًا بهلام خالٍ من العكبر، وأربع مجموعات معالجة T3، T7، T14، T21 حيث عولجت يوميًا بهلام العكبر، وتم تقييم C3 و T3 في اليوم الثالث، و C7 و T7 في اليوم السابع، وهكذا لبقية المجاميع حيث تم إجراء شقوق طولية قياس 10 × 3 مم في الحنك الصلب لجميع الحيوانات. تم قياس أقطار الجروح لمتابعة الانكماش للتقييم الشكلي. كما أُجري تقييم فوق ميكروسكوبي باستخدام المجهر الإلكتروني الماسح (SEM) لدراسة ترتيب ألياف الكولاجين، وكثافة الأورمات الليفية، وتنظيم المصفوفة خارج الخلوية، ومدى إغلاق الجرح. أظهرت الجروح المعالجة بالعكبر انكماشًا أسرع مع انخفاض ملحوظ في القطر بدءًا من اليوم السابع حيث بلغ في اليوم الحادي والعشرين قياس الجروح المعالجة ~2 مم مقارنة بـ ~5 مم في المجموعات السيطرة ($p \leq 0.0001$). وأظهرت الفحوصات النسيجية تحسينًا في التغطية الظهارية، وزيادة في تكاثر الأورمات الليفية، وترسيبًا كثيفًا للكولاجين في الجروح المعالجة. كما أكد SEM وجود ألياف كولاجين منظمة جيدًا، وعدد وفير من الأورمات الليفية، وإغلاق كامل لحواف الجرح، في حين أظهرت جروح السيطرة ترتيبًا غير محكم للكولاجين، وفجوات مستمرة، مع التهابات مستمرة.يستنتج من خلال الدراسة الحالية ان هلام العكبر بشكل ملحوظ التئام الجروح في تجويف الفم من الناحيتين الشكلية والفوق ميكروسكوبية، مما يشير إلى إمكانيته كعامل علاجي طبيعي فعال لتسريع إصلاح الأنسجة.

الكلمات المفتاحية: العكبر، الكولاجين ، الشكل ، فوق مجهري ، الجروح.