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The Effect of Topical Propolis Oil on Wound Healing: A Histological and Clinical Analysis

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ABSTRACT

Background and Objective: Propolis is a honeybee hive product composed primarily of beeswax and phytochemicals. It has been historically recognized for its antibacterial and wound-healing properties. This study aimed to evaluate the efficacy of topical propolis oil extract in accelerating cutaneous wound healing in a rabbit model.

Methods: This experimental study was conducted on twelve adult male New Zealand rabbits weighing 1.5-2 kg. The animals were divided into two groups based on healing intervals of 3 days and 7 days. Two full-thickness, 8 mm diameter wounds were created on the dorsal back of each rabbit using a sterile biopsy punch. In the control group, wounds were left to heal naturally. In the experimental group, wounds received daily topical application of propolis oil (10 µL per wound). Animals were euthanized at each time point (days 3 and 7), and wound contraction was assessed grossly. Histological and histomorphometric analyses were performed to quantify inflammatory cell counts, vascular density (blood vessel counts), and epithelial thickness.

Findings: On day three, a significant difference in wound contraction between groups was noted (control: 11.7±1.3 vs. experimental: 14.2±1.7; p=0.01). On day seven, this difference persisted (control: 41.5±8.4 vs. experimental: 54.2±8.1; p=0.02). No significant differences in inflammatory cell counts were observed on day three. However, by day seven, a significant difference was noted (p=0.001). Epithelial thickness showed significant differences on day three (control: 5.3±0.8 vs. experimental: 7.2±1.08; p=0.008) and day seven (control: 10.8±1.7 vs. experimental: 17.3±2.6; p=0.001). Blood vessel count differed significantly between groups on both days (p=0.01).

Conclusion: These findings demonstrate that topical propolis oil effectively enhances cutaneous wound healing by exerting anti-inflammatory effects and promoting cellular proliferation.

Keywords: *Propolis Oil, Wound Healing, Tissue Repair, Histological Analysis, Clinical Trials.*

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Introduction

The skin is the largest organ of the human body by surface area. It serves as the fundamental structure that protects internal tissues from extreme temperatures, UV radiation, microbial invasion, and mechanical injury (1). The wound healing process is highly complex, requiring the spatial and temporal coordination of various cell types with distinct roles in hemostasis, inflammation, proliferation, re-epithelialization, and remodeling (2). The epidermis is the outer, impermeable layer of intact skin that is exposed to the external environment. Hair follicles, sweat glands, and sebaceous glands are located within the dermis and extend through the epidermis (3).

Blood vessels, mechanoreceptors, and extracellular matrix (ECM) are abundant in the dermis, which provides the epidermis with immune defense, nourishment, and resilience. Subcutaneous adipose tissue functions as an energy reservoir and is situated beneath the dermis. It continuously provides the dermis with growth factors (4). Furthermore, the epidermis is perpetually monitored for injury by resident immune cells in each layer. To facilitate recovery following skin injury, it is necessary for a variety of cell types within the three layers to synchronize at specific intervals (5). In a sequential yet overlapping manner, hemostasis, inflammation, angiogenesis, proliferation, re-epithelialization, and remodeling occur throughout the body. Thus, cutaneous wound healing is among the most complex biological processes within the human body (6).

The antioxidant qualities of natural products may significantly contribute to the process of wound healing. The inflammatory process induces the generation of reactive oxygen species (ROS) at the wound location that can degrade proteins and harm cells. Natural products are utilized for their dual functions, specifically their antimicrobial and antioxidant properties. Honey, propolis, aloe vera, and calendula officinalis are some of the natural ingredients that are utilized in the treatment of wound-related conditions (7). Propolis is a resinous substance produced by bees. It is derived from buds, tree sap, or diverse botanical sources including poplar, birch, willow, elm, beech, alder, conifer, and horse-chestnut trees. Propolis is also occasionally referred to as bee glue (8).

Due to the fact that it possesses antibacterial and antifungal properties, propolis has been utilized in traditional medicine for millennia. Furthermore, research has highlighted several essential functions of propolis, including has therapeutic capabilities for anti-inflammation, antioxidation, and anti-tumor lesions (9). The predominant biological action of propolis originates from its bioactive constituents, including flavonoids, galangin, hydroxycinnamic acids (e.g., caffeic acid), terpenes, phenolics, and esters. In addition, propolis is abundant in a variety of vitamins and minerals, including copper, zinc, iron, cobalt, calcium, and potassium. Certain vitamins, including vitamin C, vitamin E, vitamin B, and provitamin A, are also present in propolis (10). In skin wounds, propolis helps reduce scarring and shortens the healing time. It also promotes wound contraction and accelerates tissue repair (11).

This study aims to evaluate the effectiveness of propolis oil extract in promoting wound healing in rabbits with experimentally induced full-thickness skin wounds, as assessed by histological examination.

Methods

A total of twelve male New Zealand rabbits weighing between 1.5 and 2 kg were included in this experimental study. This study was approved by the Institutional Animal Care and Use Committee of Uruk University College of Dentistry (approval number: 834; project number: 834723) on June 26, 2023. All animal procedures were conducted in strict compliance with the ethical principles of animal experimentation. The rabbits were anesthetized via intramuscular injection of 0.15 ml/kg. xylazine (12)

prior to the introduction of the wound in the right thigh. The hair on the back region was first clipped with a hair clipper, then a hair removal lotion was applied to eradicate any residual hair. A 90% ethyl alcohol solution was utilized for skin disinfection. Two wounds were created using a sterile biopsy punch with an 8 mm diameter on the dorsum of each rabbit, with depth around 1.5-2.5 mm spaced approximately 1.5-2 cm apart. The induced wound in the control group (group A) was allowed to heal naturally, while the wounds in the experimental group (group B) were treated with a daily application of topical propolis oil readymade cold pressed using a micropipette at a dosage of 10 µl. The animals were subjected to daily examinations by measuring the induced wound site with a vernier caliper to assess wound contraction. The wound closure percentage was computed with the formula: ((L1 - L2) / L1) x 100, where L1 represents the wound length on day 0 and L2 denotes the wound length on the day of observation (13). The animals were sacrificed after 3 and 7 days of healing intervals, and the tissue was fixed in 10% buffered formalin. Specimens were prepared for histological and histomorphometric analysis. To evaluate inflammatory cell infiltration, cells were counted in five high-power fields (HPF) per specimen using a light microscope (40x magnification) equipped with a square grid in one eyepiece. The mean cell count was calculated for each healing interval (days 3 and 7) (14). Epithelial thickness was measured at the wound boundaries using a 20x lens. The distance from the outermost keratin layer to the epidermal basal layer was recorded, and the mean of two measurements per specimen was calculated using ImageJ software. Blood vessel count was similarly quantified using ImageJ software. Blood vessel numerical density was measured by using a light microscope at 40x magnification in a 45 µm² area across three fields (15) and the average quantity of blood vessels was documented. The study was conducted between April and July 2024. The statistical analysis utilized SPSS (Statistical Package for the Social Sciences) software version 26, applying descriptive and inferential statistics via an independent paired T-test to compare the two groups.

Results

Histological findings:

Three days duration:

Group A (Control): Histological examination of the wound site revealed inflammatory cell infiltration, surface necrotic tissue, preserved hair follicles, numerous congested blood vessels, granulation tissue formation, and irregularly arranged collagen fibers with scattered fibroblasts (Figure 1).

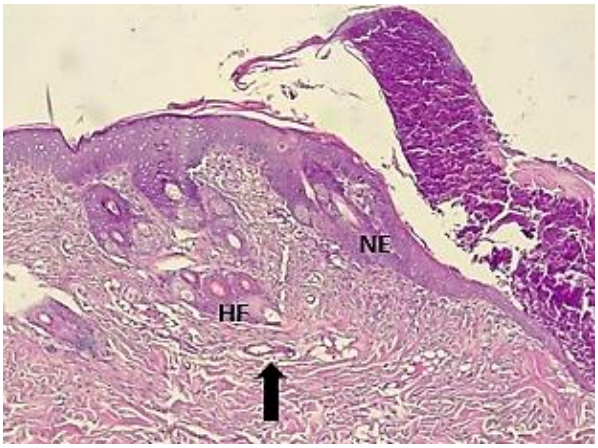


Figure 1. View of wound site showed scab (SC), new epithelium (NE), hair follicles (HF), blood vessels (BV). H&EX10

Group B (Propolis-treated): Histological analysis of the wound site after three days of topical propolis oil application revealed epithelial cell migration, residual necrotic tissue, and emergence of new hair follicles. Reorganization of collagen fibers by fibroblasts was also observed (Figure 2).



Figure 2. View of experimental group showed new epithelium (NE), inflammatory cells (arrows), collagen fibers (CF).H&Ex10

Seven days duration:

Group A (Control): Histological examination of the wound site revealed the presence of blood vessels, complete epithelialization, emergence of new hair follicles, and organized granulation tissue (Figure 3).

Group B (Propolis-treated): Histological examination revealed a superficial epithelial layer with underlying inflammatory cell infiltration. The dermis demonstrated numerous blood vessels, as well as inflammatory cells, collagen fibers, and fibroblasts (Figure 4).

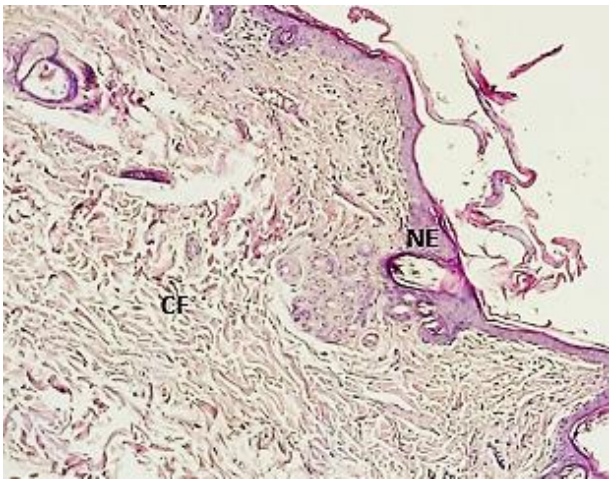


Figure 3. View of control group showed new epithelium (NE), collagen fibers (CF). H&EX10

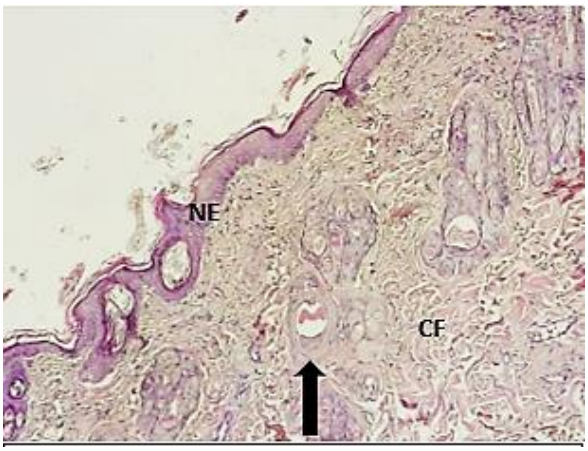


Figure 4. View of experimental group after 7 days showed new epithelium (NE), collagen fibers (CF), hair follicle (arrow).H&Ex10

Statistical findings:

Wound contraction estimation: The descriptive data for wound contraction percentage indicated that the mean values rose over time in each group, with group B demonstrating the highest values (Figure 5). A statistically significant difference between the two groups was observed at both day 3 and day 7. On day 3, the mean wound contraction was significantly greater in Group B compared to Group A (p=0.01). This difference persisted at day 7 (p=0.02), as summarized in Table 1.

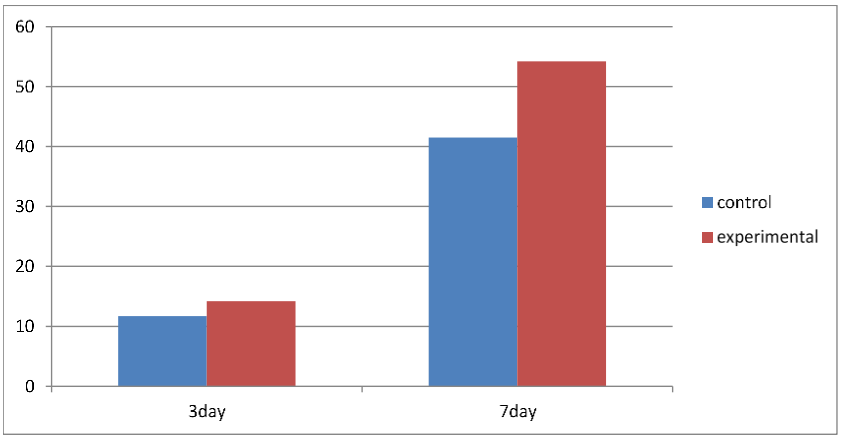


Figure 5. Mean level of wound contraction in studied groups on day 3 and day 7 after injury

Table 1. Wound contraction (%) in control and propolis-treated groups on day 3 and day 7 post-injury

Duration	Group	N	Mean±SD	Min	Max	T-Test	p-value
3 Day	Control	6	11.7±1.3	10.4	13.8	-2.82	0.01*
	Experimental	6	14.2±1.7	12.6	17.1		
7 Day	Control	6	41.5±8.4	27.9	50.8	-2.65	0.02*
	Experimental	6	54.2±8.1	41.1	63.3		

*Highly significant

Inflammatory cell parameter: Inflammatory cell counts in the propolis-treated group (Group B) decreased from day 3 to day 7. On day 3, no statistically significant difference was observed between the two groups (p=0.50). In contrast, by day 7, a statistically significant difference was observed, with Group B demonstrating significantly lower inflammatory cell counts compared to Group A (p=0.001). These results are presented in Figure 6 and Table 2.

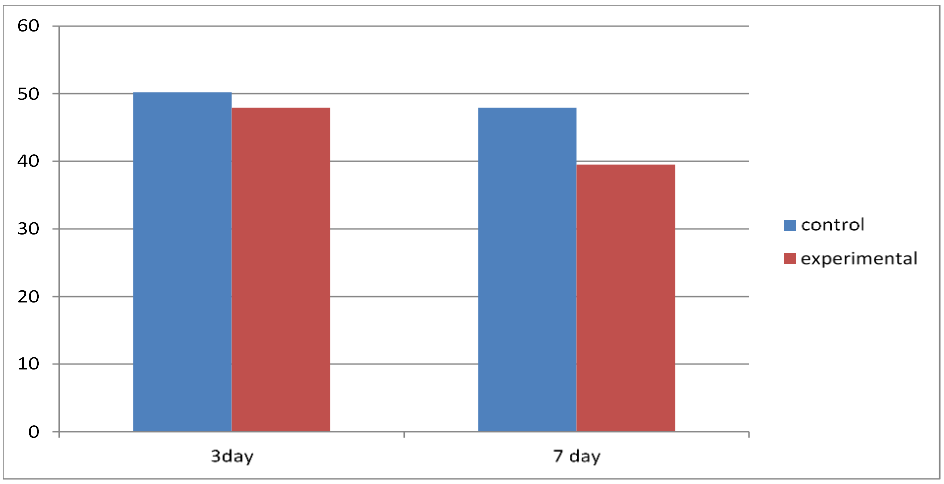


Figure 6. Mean inflammatory cell count in studied groups on day 3 and day 7 after injury

Table 2. Descriptive statistics of inflammatory cell count on day 3 and day 7 after injury

Duration	Group	N	Mean±SD	Min	Max	T-Test	p-value
3 Day	Control	6	50.2±6.1	41.9	59.3	0.6	0.5
	Experimental	6	47.9±5.4	38.8	55.3		
7 Day	Control	6	62.6±8.2	53.2	73.1	5.8	0.001*
	Experimental	6	39.5±5.2	33.6	47.9		

*Highly significant

Epithelial thickness parameter: Group B had the highest mean epithelial thickness following a 7-day healing period, whereas Group A demonstrated the lowest mean value after a 3-day healing duration. On day 3, the results showed a statistically significant difference in epithelial thickness between the two groups, with a p-value of 0.008. By day 7, the significance of the differences in epithelial thickness was even more pronounced, with a p-value of 0.001 showing a substantial disparity. The results are depicted in Table 3 and Figure 7.

Table 3. Descriptive statistics of Epithelial thickness on day 3 and day 7 after injury

Duration	Group	N	Mean±SD (mm)	Min	Max	T-Test	p-value
3 Day	Control	6	5.3±0.8	4.1	6.5	-3.3	0.008*
	Experimental	6	7.2±1.08	6.2	8.9		
7 Day	Control	6	10.8±1.7	8.2	12.9	-5.03	0.001*
	Experimental	6	17.3±2.6	13.6	19.9		

*Highly significant

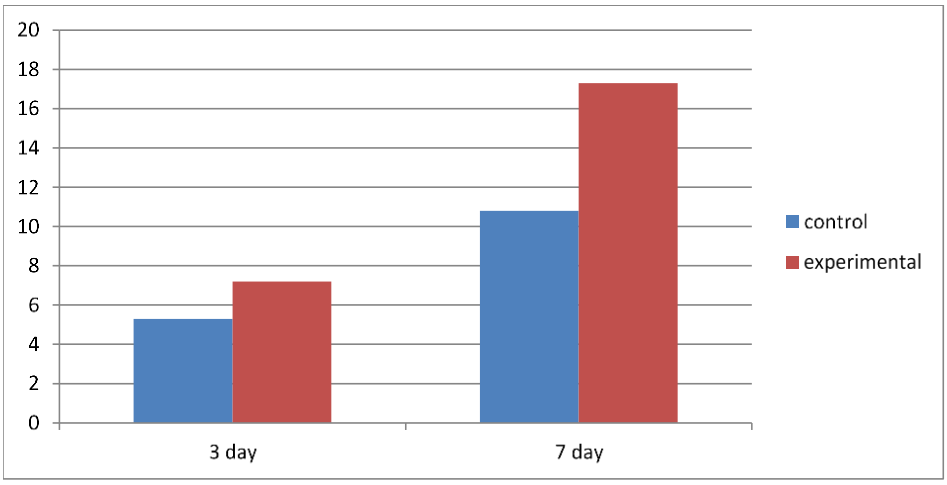


Figure 7. Mean epithelial thickness in studied groups on day 3 and day 7 after injury

Blood vessel count: The results indicated that Group B exhibited the highest mean blood vessel values over the 7-day healing period, whereas Group A displayed the lowest mean value during the 3-day healing period. On day 3, the analysis revealed a statistically significant difference in blood vessel count between the two groups, with a p-value of 0.01. Similarly, by day 7, the data continued to show a statistically significant difference in blood vessel count, with a p-value again of 0.01. These findings are also illustrated in Figure 8 and Table 4.

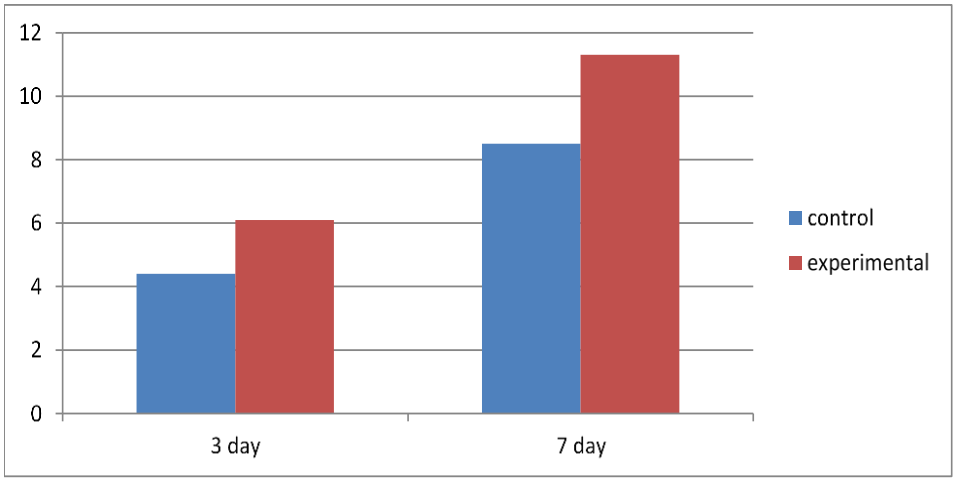


Figure 8. Mean blood vessel count in studied groups on day 3 and day 7 after injury

Table 4. Descriptive statistics of Blood vessel count on day 3 and day 7 after injury

Duration	Group	N	Mean±SD	Min	Max	T-Test	p-value
3 Day	Control	6	4.4±0.88	3.2	5.8	-2.99	0.01*
	Experimental	6	6.1±1.03	4.7	7.3		
7 Day	Control	6	8.5±0.72	7.8	9.7	-3.13	0.01*
	Experimental	6	11.3±2.06	9.2	14.6		

***Highly significant**

Discussion

The topical application of propolis oil may significantly enhance cutaneous wound healing through its anti-inflammatory properties and the stimulation of proliferative cellular activity. Specifically, propolis exhibits bioactive compounds that modulate inflammatory responses, thereby reducing edema and facilitating a conducive wound healing environment. Additionally, propolis enhances the proliferation of keratinocytes and fibroblasts, which are crucial for re-epithelialization and extracellular matrix formation (16). This dual action underscores the potential therapeutic benefits of propolis as a natural agent in the management of cutaneous injuries. Biofilm production is a significant factor in slow healing of wounds; propolis, an antimicrobial agent, can reduce the development of biofilm and expedite the healing process (17). The antimicrobial activity, along with its capacity to inhibit biofilm formation, is posited as the most significant biological property of propolis, enabling it to effectively combat diverse microorganisms, such as Streptococci, Staphylococcus aureus, Moraxella catarrhalis, and specific types of antibiotic-resistant Mycobacterium tuberculosis (18).

Previous research on excisional wound healing has demonstrated that herbal extracts, including propolis, can enhance wound repair. Specifically, propolis extract has been shown to significantly accelerate wound contraction and shorten epithelialization duration in experimental animal models compared to placebo-treated or untreated controls (19, 20). The wound healing process demonstrates a consistent and fast advancement, typically noted on the third or fourth day. Histopathological evidence indicates that propolis can expedite the inflammatory stage of the healing process for wounds by enhancing debridement activity and diminishing the volume of fibrovascular tissue, inflammatory cells, and mast cells (21). The findings indicated that propolis expedited wound closure from the onset, with significant wound contracture observed after 7 days post-injury (22).

Propolis comprises numerous chemicals with anti-inflammatory activities, such as quercetin, caffeic acid, caffeic acid phenethyl ester, naringenin, salicylic acid, apigenin, vestitol, neovestitol, ferulic acid, and galangin. These chemicals inhibit the synthesis of prostaglandins and leukotrienes, as well as the functions of myeloperoxidase, NADPH oxidase, ornithine decarboxylase, and tyrosine-protein kinase (23, 24). These bioactive constituents possess significant antibacterial, anti-inflammatory, antioxidant, immunomodulatory, and anticancer properties (25). This finding aligns with our results, which demonstrated a reduced inflammatory cell count in the propolis oil-treated group compared to the control group.

In the proliferation phase, mast cells promote specific processes, including re-epithelialization, fibroplasia, and angiogenesis. During this phase, mast cells generate substances such as TGF-β1 and VEGF, which are crucial for the proliferation of fibroblasts and the development of angiogenesis (26). By enhancing wound contraction and closure, mast cells induce the transformation of fibroblasts into a myofibroblast phenotype. Re-epithelialization is the process by which basal and suprabasal cells proliferate and migrate during the healing phase to repair a lesion (26). The current study indicates that re-epithelialization progressively increased in both the experimental and control groups. The propolis oil group exhibited a superior mean value on day seven, marginally exceeding that of the control group. This resulted from enhanced proliferation and development of epithelial cells, together with a rise in collagen fibers, fibroblast cells, and neovascularization. This is consistent with our findings. After a vigorous proliferation phase, the wound healing process enters its last remodeling or maturation phase, in which mast cells create enduring matrices essential for wound contraction (27, 28). This is also consistent with our findings, which demonstrates that epithelial thickness was greater in the group treated with propolis oil compared to the control group. Moreover, propolis and its polyphenols possess various cell-regulating characteristics. Alteration of specific epigenetic components in humans and animals is at least partially responsible for the chemopreventive effects of propolis constituents. Utilizing epigenetic modifications in cancer prevention

and treatment is logical, given their potential reversibility. The impact of propolis on metabolic alterations requires further investigation, particularly given the influence of histone acetylation on tumor metabolism and the potential role of propolis polyphenols in modulating epigenetic activity. Future research should concentrate on the mechanisms through which propolis and its constituents influence epigenetics, as well as the prospective application of these alterations in the formulation of novel pharmacological approaches for effective cancer prevention and chemopreventive agents (25). Despite extensive research efforts, propolis- and honey-derived bionanomaterials have achieved only limited success in obtaining regulatory approval for medical applications. The following factors are responsible for this limited success: (1) the complexity of the wound healing process; (2) the unpredictable behavior of innovative bionanomaterials; (3) limited study models, particularly for chronic wounds; (4) inadequate understanding of the long-term toxicity of prolonged exposure to novel therapies; and (5) the potential impact of these therapies on the emergence of microbial resistance (29).

Propolis possesses significant potential in wound healing and tissue regeneration by enhancing wound contraction, accelerating re-epithelialization, exerting anti-inflammatory effects, and promoting angiogenesis.

Conflicts of interest: The authors have no relevant affiliations or financial interests with any organization or entity that would present a conflict of interest.

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