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Article:

The Use of Propolis Nonwoven Material Sheets (NMS) to Enhance Wound Healing in Rabbits: A Macroscopic and Histopathological Evaluation

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Abstract

Propolis, a resinous substance that bees gather from plant exudates, buds and combine with wax and bee enzymes, has biological qualities that make it a great choice for pharmaceutical and medical applications. These qualities include anti-inflammatory, antimicrobial, antioxidant, immunomodulatory, and analgesic effects. This study investigated the efficacy of propolis nonwoven material sheets (NMS) in accelerating wound healing in rabbits. Twenty clinically healthy native rabbits underwent surgery to create 1 × 1 cm² excisional wounds on the thoracic region at the right side of their backs. Rabbits were divided into two groups: a Propolis-treated group given Propolis NMS sheets as wound dressings and a control group received normal saline. Both groups were evaluated macroscopically and histopathologically at 7-, and 14-days post-wound induction. The results indicated that propolis NMS significantly enhanced wound healing, evidenced by improved re-epithelization and reduced wound size. By day 14, wounds treated with propolis showed a complete epithelial closure, whereas control wounds were slower to heal and exhibited scabbing. The propolis-treated wounds had fewer inflammatory cells, more collagen, and fibrin, and increased fibroblast and capillary density compared to the control group. These results suggested that propolis promotes granulation tissue formation and wound organization, making it a valuable material for managing large open wounds in animals. In conclusion, propolis NMS accelerates wound healing and is recommended for clinical use due to its effectiveness and ease of application.

Keywords: Collagen, Microscopical Estimation, Nonwoven Materials, Propolis, Wound Healing.

Introduction

Damage or disruption to normal anatomical structure and function is known as a wound [1]. It can be as simple as a break in the skin's epithelial integrity or it can be more extensive, extending into subcutaneous tissue and causing damage to other structures like muscles, tendons, vessels, nerves, parenchymal organs, and even bone [2]. Pathological processes that start either internally or externally within the affected organ can result in wounds. They may be the consequence of a disease process or have an unintentional or deliberate cause. Any type of wound, regardless of its origin, destroys the tissue and alters its internal environment. Bleeding, vascular constriction with coagulation, complement activation, and inflammatory response are physiological reactions to the noxious factor [3, 4].

Several coordinated processes, such as bleeding, coagulation, the initiation of an acute inflammatory response to the initial injury, the regeneration, migration, and proliferation of connective tissue and parenchyma cells, the synthesis of extracellular matrix proteins, the remodeling of new parenchyma and connective tissue, and collagen deposition, are all part of what is known as normal wound healing [5, 6]. Ultimately, a systematic process of

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improving wound strength leads to the healing of damaged tissues [5, 7]. Awareness of the healing process and the characteristics of the different dressing materials is essential for effective wound management to optimize treatment effectiveness [8]. Traditional and modern therapies are two basic categories into which wound healing therapies can be divided. The trend currently shifts to creating novel wound care products that combine modern goods and traditional healing agents, such as hydrogel sheets containing honey, aloe vera loaded into alginate hydrogels, and nanofibers containing silver nanoparticles [9-11].

Propolis originates from the Greek words "pro" which means "the entry of" and "polis" which means "the city" [12], accurately characterizing this bee product, which protect the beehive entrance. Bees make propolis, a sticky, dark-colored resinous substance, to line the inside of their colonies. Depending on the kinds of plants that bees may reach, propolis composition varies greatly throughout sources [13]. To this point, the propolis season of harvesting, geographic location, and plant origin have an impact on its chemical structure [14]. Another crucial element in identifying the chemical and biological components of propolis is the breed of bee [13]. Physically, Propolis can range in color from yellow-green to brown, depending on its age and place of origin [15]. Propolis is made up of 50% resin, 30% wax, 10% oils, 5% pollen, and other phenolic ingredients like flavonoids [16]. Butyric acid, minerals, vitamins A, B, C, D, and E, glucose, and amino acids are also included in propolis [17]. Propolis's advantages for health and medical concerns are closely linked to its biological functions. Propolis serves a wide range of biological purposes, including antibacterial [18], antidiabetic [19-21], anticancer [22, 23], anti-inflammatory activities [24, 25], antiulcer [26], antioxidant [27], and antitumor properties. These various benefits highlight propolis's importance in wound healing [28].

The use of nonwoven materials sheets (NMS) to protect against biological agents is common in medical applications. They prevent the spread of infections and cross-contamination by providing safety features including resistance to illnesses and infections. To reduce handling and the spread of contamination, NMS are used only once before being burned. Shorter manufacturing cycles, greater flexibility, and cheaper prices are the reasons behind their popularity [29]. Nonwovens have several advantages for wound dressings, including ease of fabrication, air permeability, non-adherence, high absorbency, a large surface area, dust-free, and a surface mechanism that may be readily conditioned to work as an effective dressing material [30].

Propolis has been suggested as a material that promotes wound healing, but reports of the application of propolis nonwoven sheets (Propolis NMS) as dressings for wound care are lacking[31, 32]. Thus, the purpose of this study was to assess the healing of rabbits' complete cutaneous excisional wounds covered with propolis NMS covering.

Materials and Methods

The experiment was conducted as a prospective study at the Department of Surgery, Anesthesiology, and Radiology, within the Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Sohag University, Sohag, Egypt. The Faculty of Veterinary Medicine, Sohag University's veterinary medical research ethics committee approved the animal study and all experimental procedures. These approvals comply with OIE standards for using animals in research, under the reference number Soh.un.vet/0005 M.

A total of twenty healthy Native adult male rabbits ranging from 3-6 months old and 1-2 kg in weight were used in the current study. All rabbits were housed individually in galvanized steel cages at the Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Sohag University with feeding twice daily with a standard balanced diet and water (adlibtum), temperature controlled at 22° ± 2°C with controlled photoperiod 12 hours light/dark. An acclimatization period of 7 days was performed before the experiment. All rabbits were dewormed, protected against external parasites, and vaccinated against Rabbit Viral Hemorrhagic Disease and Rabbit Hemorrhagic Septicemia before the operation. Rabbits underwent surgery to create 1×1 cm^2 excisional wounds on the thoracic region at the right side of their backs, and then rabbits were randomly divided into two groups: a treated group that was given Propolis NMS sheets as wound dressings (N=10) and a control group that received no treatment (N=10).

Depending on the desired film thickness, a suitable amount of the solution (2g of propolis powder dissolved in 5 ml of ethanol) was spin-coated by using (VTC-50 A) on the substrates (5cm × 5cm NMS sheets) at an appropriate speed of 1500- 2500 rpm by using spin coating technique (Spin coating uniformly deposits thin coatings over substrates using a high-speed spin, affecting the thickness of the thin-film based on solvent, viscosity, concentration, and spinner angular speed) [33].

Propolis sheets were prepared with the aid of a physicist at the physical labs of the faculty of science at Sohag University, Egypt.

1. Experimental study

Rabbits were starved for 8 hours before surgery. Rabbits were anesthetized by intramuscular injection of a mixture of xylazine–ketamine hydrochloride (Xylazine: 5mg/kg B.W and Ketamine: 35mg/kg B.W) administered in one syringe. The operated rabbits were restrained in sternal recumbency on the surgical board, and the surgical field (back at the thoracic region) was prepared for aseptic surgery; clipped, shaved, scrubbed by povidone-iodine and, disinfected with alcohol 70%, covered with sterile towel exposing dorsal side only (the wound creation site).

Using a sterile template, a 1×1 cm2, the full cutaneous excisional wound was created using a scalpel and scissors on the thorax region. By back pressure with a sterile tampon hemostasis was achieved. Wound measurements were calibrated using a digital caliper (length, width, depth) and were digitally photographed. The Propolis treated group (N=10) was covered with prepared propolis sheets, and gauze, and was fixed by silk plaster; while the control group (N=10) was irrigated with normal saline and was covered by sterile pad dressing, and gauze, and was fixed by silk plaster (Figures 1, 2).

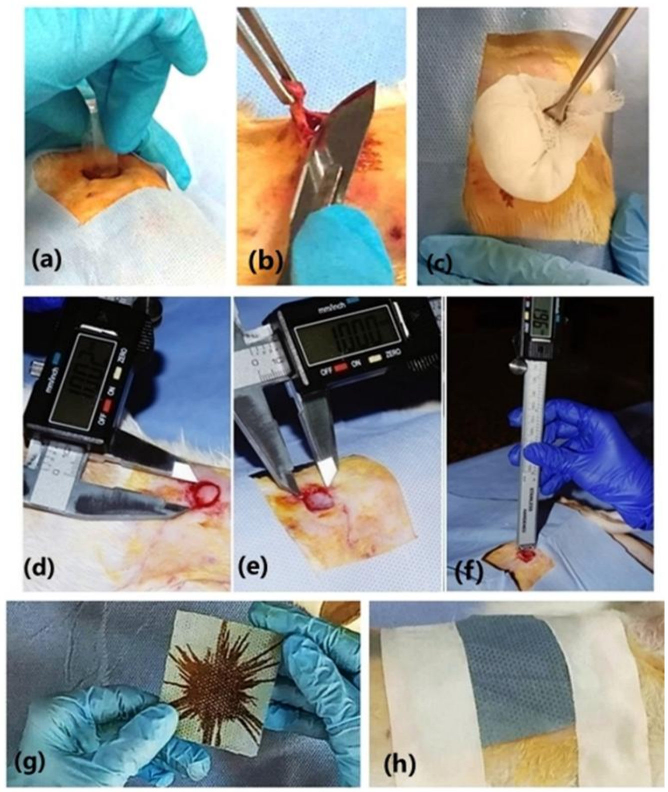

*Figure 1:(a) 1*1cm2 template (b) skin removal for wound creation (c) tampon to stop bleeding (d) digital calibration of length (e) digital calibration of width (f) digital calibration of depth (g) propolis film (h) covered the wound with propolis sheet*

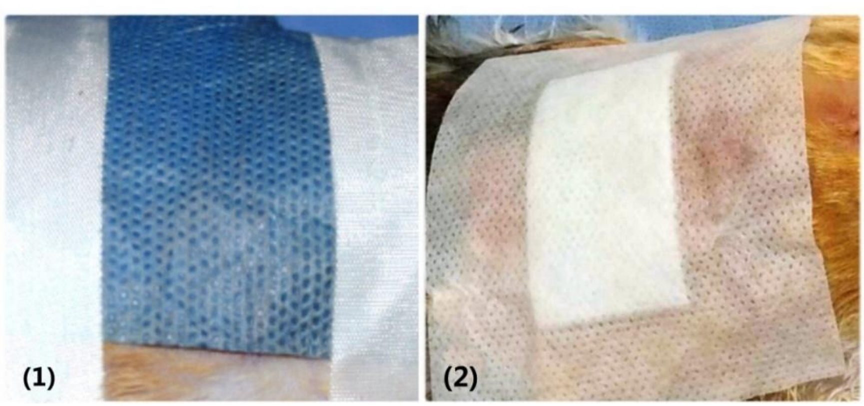

Figure 2: (1) propolis group covered with prepared propolis sheets, gauze, and fixed by silk plaster, (2) control group covered by sterile pad dressing, and gauze, and fixed by silk plaster.

Rabbits were allowed to move freely in the cages postoperatively throughout the experiment. They were given a single dose of antibiotic directly after the surgery. Rabbits were under clinical observation, and wound dressings were changed twice a week (3-day intervals).

2. Evaluation of the study

All groups were evaluated at 7, and 14 days postoperatively for clinical and histopathological evaluation.

2.1 Clinical evaluation

2.1.1 Gross evaluation

At each wound evaluation interval (7-, and 14-day post-wound induction), wounds were examined grossly for the presence any abnormal signs (e.g. infection, exudates, or even exuberant granulation tissues; and examined for changes in wound coloration).

2.1.2 Planimetry of wounds

Wounds were photographed on days (0, 7, and 14) post-wound induction and wound measurements were calibrated using a digital caliper (length, width, depth). Photos were taken after focusing on the shape of the wounds. The percentage of the wound size (*The rate of wound contraction*) was measured to calculate the percentage of the wound contraction depending on the equation described by Ramsey et al [34].

Wound Contraction (WC) =

$$(WC\ \%) = (W0 - WI) / W0 \times 100$$

Where: W0 = the initial wound surface area measurement (1st measurement in cm), WI = the wound surface area measurement on the day of the measurement (2^{nd} measurement in cm).

Wound volume was measured by using (digital caliber), According to Kundin's mathematical formula to estimate the wound volume (V) through its surface area (A), using the two largest dimensions: length (L), width (W), and depth (D) using the following equation [35]:

$$V = A \times D \times 0.327, \text{ where } A = L \times W \times 0.785$$

2.2 Histopathological evaluation

The animals of each group were euthanized at 7, and 14 days postoperatively, and tissue specimens were taken from wounds to be evaluated histopathologically. Skin samples were collected from rabbits and were fixed in (10%) neutral buffered formalin solution; Skin wounds of all animals in the experimental groups were dissected, and quickly fixed, processed through the conventional paraffin embedding technique [37], and then, sections were dewaxed with xylene and rehydrated through a descending alcohol series. Slides were stained with Harris hematoxylin and eosin (H & E) [36, 37] and Masson's Trichrome stains (specific stain for collagen content)[38, 39]. All sections were examined and were photographed by using a light microscope OLYMPUS CX43 microscope then photographed with an OLYMPUSDP72 camera adjusted to the microscope (Department of Pathology and Clinical Pathology, Faculty of Veterinary Medicine, Sohag University).

3. Statistical analysis

Data from all experimental groups were presented as mean ± standard deviation (SD) and analyzed using GraphPad Prism Version 5 (San Diego, California, USA). Statistical analysis included the Mann-Whitney U test, one-way ANOVA with Tukey's post-hoc multiple comparison tests, and two-way ANOVA with Bonferroni post-test to compare replicate means by row, as appropriate for the required test. Significance was set at $p<0.05$ [40, 41].

Results

1. Clinical evaluation

During the entire experiment, the rabbits were conscious, showing normal behavior, and having regular access to both food and water. Deaths were not reported.

1.1. Gross evaluation of wounds:

On day 7 post-wound induction, granulation tissue began to cover the wounds on propolis-treated and control wounds. Propolis NMS treated wounds had no volume at the end of the first week because the depth had filled in completely, whereas the control groups had superficial depths and. it was additionally observed that propolis-treated wounds showed normal odor with no exudation, while some control cases had pus and an unpleasant odor (Figure 3, 4).

On day 14 post-wound induction, the propolis-treated wounds gave signs of healing, without any exudation or smell, and there was less granulation tissue, while; hypergranulation tissues and a trace amount of suppurative exudate were visible in the control wounds (Figure 3, 4).

Figure 3: represents the gross evaluation of propolis groups at day (0-, 7-, and 14) post-wound induction.

Figure 4: represents the gross evaluation of control groups at day (0-, 7-, and 14) post-wound induction.

1.2 Planimetry of wounds:

Results revealed a significant increase in the contraction rate over time (p<0.0001) in propolis NMS-treated and control groups, there was a significant difference between the propolis-treated and control groups (p<0.05) after 7 days compared with that after 14 days,

On day 7 post-wound induction, the wound size reduction percentage showed a significant difference in groups (p < .01). And the Wound contraction rate was higher in Propolis treated group (36.97%) than in the control one (22.01%) (Figure 5, 6).

On day 14 post-wound induction Propolis-treated wounds exhibited a no significant reduction in wound size compared to control ones. On the other side, the wound contraction rate was (97.06% and 89.49%) for the propolis-treated group and control group, respectively (Figure 5, 6).

Table 1: summarizes the mean reduction in the wound width and length as well as the mean wound contraction percentage.

Substances		After 7 days	After 14 days
Propolis	Dimensions (cm) (LxW)	9.018 ± .199 × 7.14 ± .287	2.236 ± .597 × 1.258±.568
	WC%	36.97	97
Control	Dimensions (cm) (LxW)	10.5 ± .711 × 9.068 ± .556	2.902 ± .213 × 2.62 ± .158
	WC%	22	94.36
P value between the two groups		(p < .01)	(P > 0.05)

Length and width values are expressed as Mean ± SE, values indicated by different letters

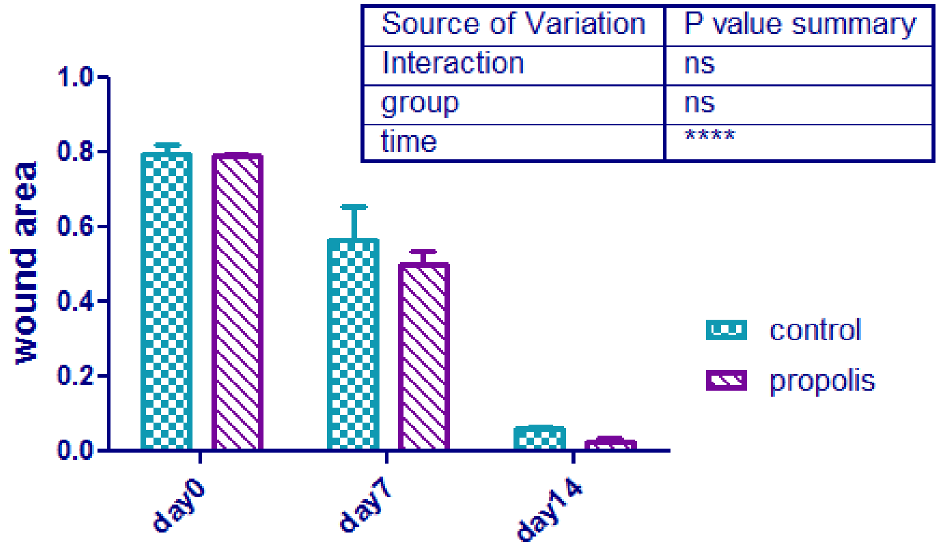

Figure 5: Propolis and control wounds area planimetry at 7 and 14 days after wound induction in rabbits, p value (P > 0.05), non-significant at days 7 and 14.

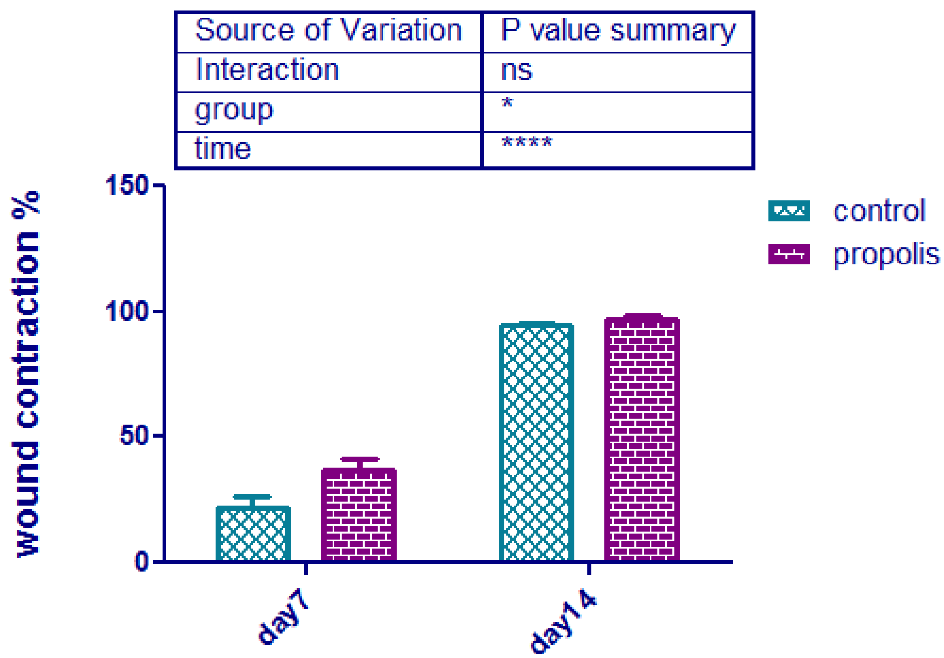

Figure 6: Propolis and control wounds contraction rate planimetry at 7- and 14 days after wound induction in rabbits, p value (P < 0.01) was significant at day 7, while (P > 0.05), non-significant at day 14.

2. Histopathological examination

After 7 days post-wound induction, skin tissue samples from the control group showed a large scab over necrotic tissue, with inflammatory edema. Development of granulation tissue with new capillaries at the wound site was observed, along with marked mononuclear inflammatory cellular infiltration. Mild collagen production in the granulation tissue and mild development of elastic fibers in the dermal layer were also noted (Figure 7).

In contrast, skin tissue samples from the propolis-treated group after the first week post-surgery exhibited a necrotic tissue layer at the wound site, inflammatory edema, and the development of granulation tissues characterized by angiogenesis and fibrogenesis. Mild production of collagenous fibers in the dermal layer and mild development of elastic fibers, mainly present in the vascular elastic lamina in the dermal layer were also demonstrated (Figure 8).

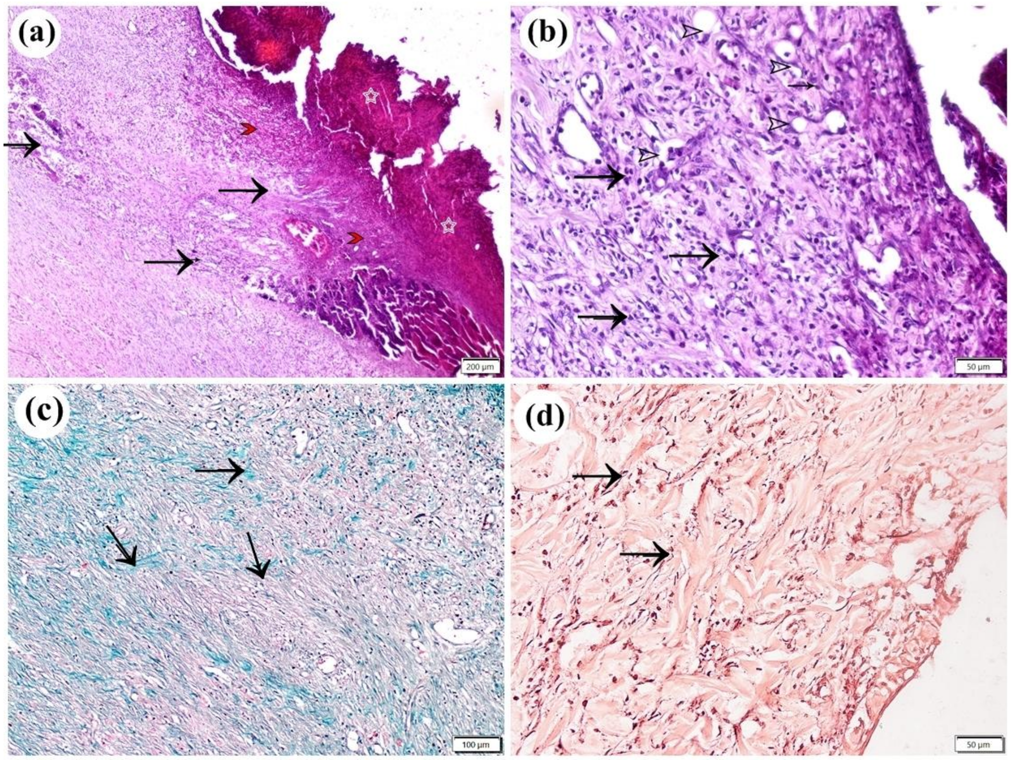

Figure 7: Representative photomicrographs of skin tissue samples from the control positive (untreated) group after the 1st week of surgery (a,b) stained by hematoxylin and eosin (H&E) demonstrate large scab (stars) over necrotic tissue (arrowheads), with inflammatory edema (arrowheads). Development of granulation tissue with the formation of new capillaries (arrowheads) at the site of skin wound, marked mononuclear inflammatory cellular infiltration (arrows). (c) Stained by Masson's trichrome stain demonstrate mild collagen production in granulation tissue (arrows). (d) Stained by Orcein stain demonstrates mild development of elastic fibers in the dermal layer; at scale bar (a) 200µm, (b, d) 50µm, (c) 100µm.

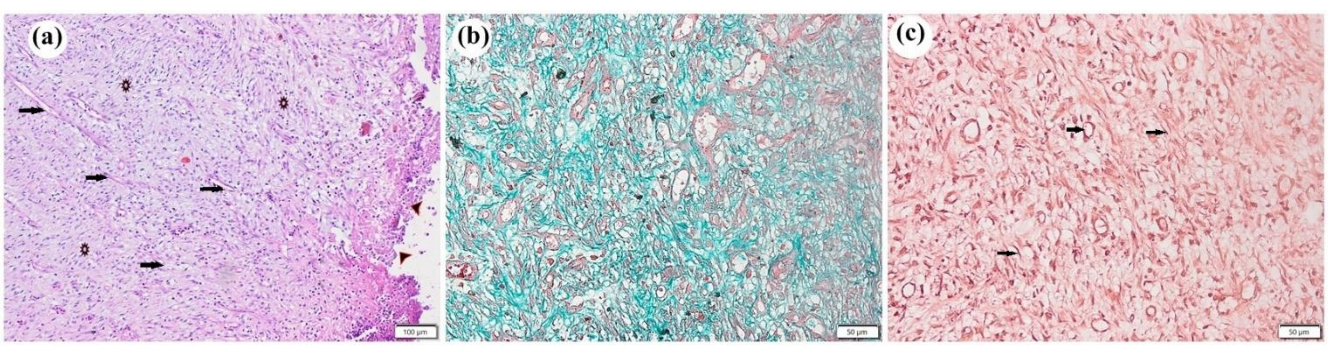

Figure 8: Representative photomicrographs of skin tissue samples from the propolis-treated group after the 1st week of surgery, (a) stained by hematoxylin and eosin (H&E) demonstrates necrotic tissue layer on the wound site (arrowheads), with inflammatory edema, development of granulation tissue characterized by angiogenesis (arrows), and fibrogenesis (stars). (b) Stained by Masson's trichrome stain demonstrates mild production of collagenous fibers (blue color) in the dermal layer. (c) Stained by Orcein stain demonstrate mild produced elastic fibers mainly present in vascular elastic lamina in the dermal layer; at scale bar (a) 100µm, (b, c) 50µm.

After 14 days post-wound induction, skin tissue samples from the control group still showed loss of the epithelial layer with the development of granulation tissue formation and angiogenesis (multiple new capillaries) accompanied by intense perivascular inflammatory cellular infiltration and fibrogenesis with disoriented fibers. There was moderate collagen production in the granulation tissue and mild development of elastic fibers in the dermal layer (Figure 9).

In contrast, skin tissue samples from the propolis-treated group demonstrated mild re-epithelialization, inflammatory cellular infiltration with moderate edema, moderate development of hair follicles, and a moderate arrangement of collagenous bundles. There was also moderate production of collagenous fibers with more evenly distributed elastic fibers in the dermal layer (Figure 10)

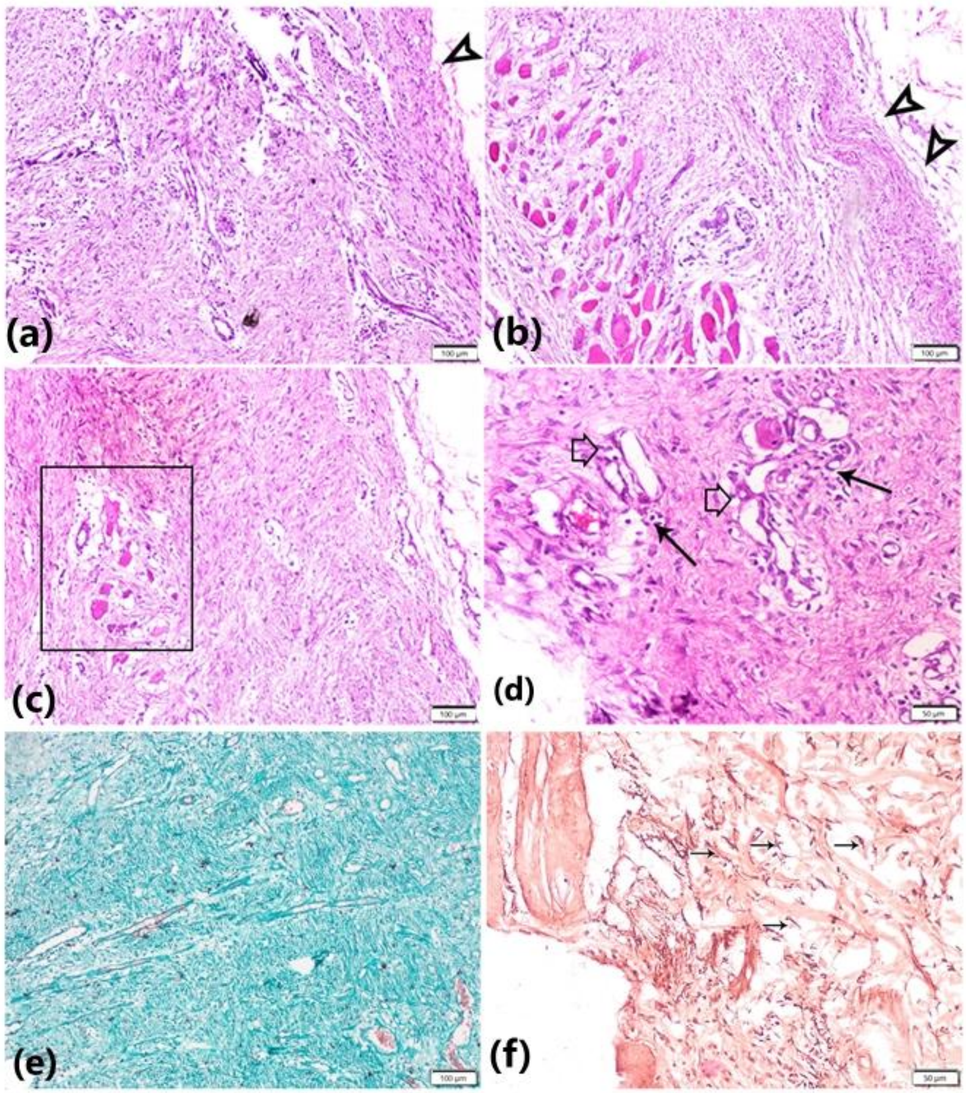

Figure 9: Representative photomicrographs of skin tissue samples from the control positive (untreated) group after the 2nd week of surgery, (a-d) stained by hematoxylin and eosin (H&E) demonstrate (a & b): loss of epithelial layer with granulation tissue formation (arrowheads), (c selected rectangle magnified in d): angiogenesis (multiple new capillaries) (arrowheads) with intense perivascular inflammatory cellular infiltration, fibrogenesis with disoriented fibers (arrows). (e) Stained by Masson's trichrome stain demonstrates moderate collagen production in granulation tissue. (f) Stained by Orcein stain demonstrates mild development of elastic fibers in the dermal layer; at scale bar (a, b, c and e) 100µm, (d, f) 50µm.

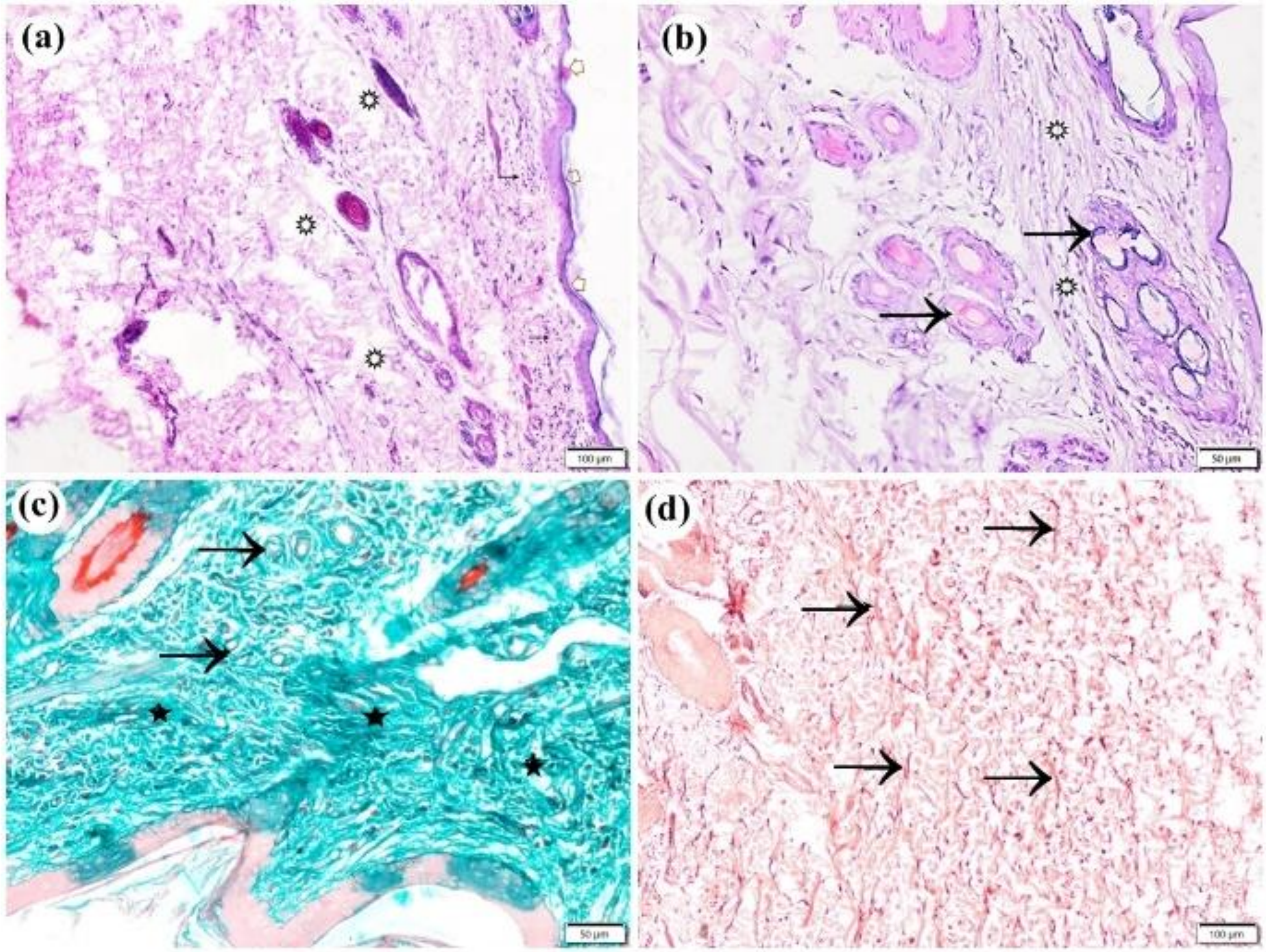

Figure 10: Representative photomicrographs of skin tissue samples from propolis treated group after the 2nd week of surgery, (a,b) stained by hematoxylin and eosin (H&E) demonstrate mild developed re-epithelialization (arrowheads), inflammatory cellular infiltration (arrows), moderate edema (stars). Moderately developed hair follicles (arrows), and moderately arranged collagenous bundles (stars). (c) Stained by Masson's trichrome stain demonstrates moderate production of collagenous fibers (blue color) in the dermal layer. (d) Stained by Orcein stain demonstrates more or less distributed elastic fibers in the dermal layer; at scale bar (a, d) 100µm, (b, c) 50µm.

Discussion

Interactions between various cell types, coagulation factors, and growth factors are essential to the wound-healing process [42-44]. To restore tissue integrity, wound healing goes through phases that are triggered by intracellular and intercellular pathways. Hemostasis, inflammation, proliferation, and remodeling are some of these stages [45-49].

Chronic wounds can result from any impairment throughout the healing phases, which may have an effect on one's quality of life [43]. Persistent wounds increase morbidity and even mortality, as well as length of hospital stay [50]. Treatment and management practices that restrict wound healing are primarily linked to chronic wounds [51]. As a result, numerous investigations are carried out to lower expenses and develop more efficient wound therapies [52].

Propolis has been shown to have antiviral, antibacterial, antiprotozoal, and antifungal properties in numerous investigations [53-56]. As a result, antimicrobial ingredients may be useful in quickening the healing process. There is currently proof that propolis has special antibacterial properties that can be used to treat burn injuries and diabetes [57, 58]. Wojtyczka et al demonstrated that propolis inhibits the growth of bacteria and stops the production of biofilms [59].

Applying propolis topically has the potential to improve wound healing by lowering mast cell numbers, which accelerates the healing process[60]. The active chemicals in propolis, including caffeic acid phenethyl ester, may be the reason for the notable decrease in mast cells in the surgical site during the initial inflammatory phase [60].

The antimicrobial activity of propolis can be influenced by several variables, including the sample's dose, source, and extraction solvents [61]. Propolis has demonstrated minimal action against gram-negative bacteria, but it is effective against gram-positive bacteria, spores, fungi, and viruses, according to several studies [62-64].

Propolis's physicochemical characteristics make it beneficial for wound closure. The effect of propolis on the healing of cutaneous wounds in adult mongrel dogs was assessed in a 2015 study. Fibroblasts, keratinocytes, endothelial cells, and inflammatory cells are all involved in cutaneous wound healing. Propolis paste has been shown to enhance dogs' cutaneous wound healing in a time-dependent way. Propolis' immunomodulatory, antibacterial, antioxidant, analgesic, and anti-inflammatory properties are thought to be responsible for the accelerated wound healing [28]. In 2005, a study comparing the effects of propolis and silver sulfadiazine on burns in Wester Albino rats in Turkey discovered that topical propolis administration effectively controlled the infection while producing clean granulation tissue [65].

Nonwoven materials (Nms) are widely utilized in the medical industry and in other fields to protect against biological agents. When it comes to material qualities and user needs, Nms for medical applications are particularly noteworthy for the many benefits they provide [29]. Over the last few years, key technologies for creating NMs for medical purposes have included melt-blowing, spun-bonding, hydro-entangling, dry-laid, carding and air-laying, needle-punching, and thermal bonding [66].

Other unique qualities include high absorbency and non-adherent compress surfaces, in addition to comfort and skin-friendly qualities. Since blood and fluids are transferred to the absorbent pad due to the unique fiber structure, compresses are therefore perfect for the treatment of either dry or secretary wounds [67]. Nonwovens are favored because sterilization is a critical concern for surgical dressings. Generally speaking, nonwovens can be lint-free and smooth. Debris is less likely to remain in the wound as a result. The use of latex or thermally calendaring can soften and increase the absorbency of nonwovens [68]. Nonwovens can be custom-made with any desired attribute, including breathability, homogeneity, and superior barrier qualities.

Nonwovens are used for the backings and wound paddings in surgical dressings, for example, because of their unique properties, which include ease of processing, air permeability, non-adherence to wounds, high absorption, a large surface area free of debris, and surface mechanisms that can be easily conditioned to function as a great dressing material [30].

The physiochemical and biological characteristics of NMS, along with propolis's significant roles in antibacterial, antioxidant, anti-inflammatory, wound healing, immunomodulatory, antitumor, and anti-proliferative activities, make Propolis NMS dressings an effective way to overcome wound healing challenges [56, 69].

Our results showed that Propolis NMS promoted dermal healing that began from the wound borders toward the center, reducing the size of the wound by favorably enhancing the re-epithelization of the wound region. Conversely, the control wounds' rate of healing decreased as they were beyond the scab.

Propolis NMS-treated wounds' exhibited a necrotic tissue layer at the wound site with inflammatory edema, by the second week it showed inflammatory cellular infiltration (macrophages, neutrophils) with moderate edema. These cells are crucial for Eliminating microorganisms and dead cells and cleansing up the wound site [70, 71]. By the third week after the wound's induction, the number of inflammatory cells had gradually decreased. On the other hand, control wounds showed a large scab over necrotic tissue, with inflammatory edema and marked mononuclear inflammatory cellular infiltration.

Propolis-treated wound at the end of the first week showed fibrogenesis with mild collagen production in granulation tissue which by the second week became moderate and by the time showed significant collagen production in granulation tissue [72] with moderate development of elastic fibers in the dermal layer and marked development of hair follicles, and sebaceous gland. On the other hand, control wounds showed disoriented collagen bundles, mild elastic fibers, and mild development of hair follicles.

The formation of new blood vessels at the wound site is crucial to the healing process because it provides the area with oxygen, nutrition, and immune cells. In addition, they remove harmful waste [73, 74]. Comparing wounds treated with propolis NMS to control ones, the first one showed an increase in the quantity and size of newly created blood vessels, which aided in the healing process.

Conclusion

1) Medicinal propolis helps speed up the healing of wounds.
2) Healing enhancement is shown through reduction of the duration of the healing phases.
3) Propolis encourages re-epithelialization in wound healing.

4) Because propolis NMS has a clinically significant effect and is simple to apply, it is recommended that it applies as a wound dressing to hasten the healing process.

Conflict of interest

The authors declare that they have no conflict of interest.

Author's contributions:

All authors contributed equally to the manuscript. All authors wrote, assisted with editing the manuscript, and approved the final version.

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