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Therapeutic properties of Algerian propolis in skin wound healing with significant tissue loss

Propiedades terapéuticas del própolis Argelino en la cicatrización de heridas cutáneas con pérdida significativa de tejido

Abdellatif Boudra*[1], Rachid Merati [1], Berrani Abdelkader[2], Wassim Yezli [2], Helali Hadil[2], Imen Henni [2]

[1]University of Tiaret, Laboratory of Hygiene and Animal Pathology. 14000, Tiaret, Algeria.
[2]University of Tiaret, Faculty of Natural and Life Sciences. 14000, Algeria.
Corresponding author: abdellatif.boudra@univ–tiaret.dz

ABSTRACT

This study investigates the therapeutic properties of Algerian propolis in skin wound healing with significant tissue loss. It includes an *in vitro* phase to formulate an ethanolic extract of Algerian propolis (EEPA) ointment and evaluate its antimicrobial activity, and an *in vivo* phase to compare its effects with silver sulfadiazine cream and a control group in rabbit wound healing. *In vitro*, two propolis samples (P1 and P2) were tested against *Staphylococcus aureus*, showing inhibition zones of 9.5 ± 1.04 mm (P1) and 11.8 ± 0.65 mm (P2). *In vivo,* the propolis–treated group (PG) achieved complete wound closure (16 cm^2) within 16–30 days (d), compared to 24–36 d for the silver sulfadiazine group (SDG), while the control group (CG) did not achieve full closure after 36 d. Healing scores were highest in PG (2.27 ± 0.59 to 2.80 ± 0.46) and dressing evaluation scores were also superior (2.5 ± 0.51). Faster fur regrowth was observed in PG, enhancing wound aesthetics. Postoperative hypothermia affected all groups, with CG experiencing the greatest temperature drop (>2°C) and higher mortality. In conclusion, Algerian propolis, particularly P2, exhibited superior activity due to its higher polyphenol content, demonstrating its potential as a natural antimicrobial agent shows strong potential in wound management by enhancing antimicrobial protection, accelerating healing, and improving wound aesthetics. Further studies are needed to optimize its application and elucidate its mechanisms of action.

Key words: Propolis; wound healing; antimicrobial properties; *in vitro* study; *in vivo* study

RESUMEN

Este estudio investiga las propiedades terapéuticas del propóleo argelino en la cicatrización de heridas cutáneas con pérdida significativa de tejido. Incluye una fase *in vitro* para formular una pomada a base de extracto etanólico de propóleo argelino (EEPA) y evaluar su actividad antimicrobiana, y una fase *in vivo* para comparar sus efectos con la crema de sulfadiazina de plata y un grupo de control en la cicatrización de heridas en conejos. En la fase *in vitro*, se probaron dos muestras de propóleo (P1 y P2) contra *Staphylococcus aureus*, mostrando zonas de inhibición de 9,5 ± 1,04 mm (P1) y 11,8 ± 0,65 mm (P2). En la fase *in vivo*, el grupo tratado con propóleo (PG) logró el cierre completo de la herida (16 cm^2) en 16 a 30 días (d), en comparación con los 24 a 36 d del grupo tratado con sulfadiazina de plata (SDG), mientras que el grupo control (CG) no logró un cierre completo después de 36 d. Los puntajes de cicatrización fueron más altos en PG (2,27 ± 0,59 a 2,80 ± 0,46), y las evaluaciones del apósito también fueron superiores (2,5 ± 0,51). Se observó un crecimiento más rápido del pelaje en PG, mejorando la estética de la herida. La hipotermia postoperatoria afectó a todos los grupos, siendo más pronunciada en CG (>2°C) con mayor mortalidad. En conclusión, el propóleo argelino, especialmente P2, mostró una actividad superior debido a su mayor contenido de polifenoles, lo que demuestra su potencial como agente antimicrobiano natural muestra un gran potencial en el manejo de heridas al mejorar la protección antimicrobiana, acelerar la cicatrización y optimizar la estética de la piel. Se requieren más estudios para optimizar su aplicación y esclarecer sus mecanismos de acción.

Palabras clave: Propóleo; cicatrización de heridas; propiedades antimicrobianas; estudio *in vitro*; estudio *in vivo*

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INTRODUCTION

The skin is an organ essential for the survival of the human body, providing protection against environmental influences. It is therefore important to maintain its physiological properties to avoid affecting the body's homeostasis. However, its integrity can change in various ways throughout life (burns, cuts, tears, among others.), triggering the healing process to replace tissue loss, allowing the skin to regain its barrier role [1].

The healing of a skin wound involves an extraordinary mechanism of cascading cellular functions, unique in nature. Wound healing is a complex series of reactions involving overlapping processes, including the induction of an acute inflammatory process, regeneration, migration, and proliferation of parenchymal and connective cells, synthesis of extracellular matrix proteins, as well as remodeling of connective tissue. This leads to the formation of scar tissue in parallel with wound contraction and epithelialization [2].

Intercellular communication between growth factors (GF) and cytokines plays a crucial role in guiding this process, as these signaling molecules are released and contribute to the healing cascade. In response to tissue injury, inflammatory and other stromal cells are recruited to the wound site [3].

The use of propolis in traditional medicine has been supported since antiquity for its therapeutic, anti–inflammatory, gastrointestinal, and other benefits. It also has protective, anti–tumoral, and anti–diabetic properties [4]. Propolis is one of the six bee products, along with honey, royal jelly, pollen, wax, and bee venom. This resinous, balsamic, and gelatinous substance is collected by a highly diverse population of bees (*Apis mellifera*). Plant sources combined with compounds are produced from bee secretions, wax, and saliva [5]. Bees use propolis as an antibiotic against foreign organisms and to repair cracks in the hive [6].

The chemical composition of propolis varies depending on the geographical area, time of collection, seasonality, illumination, altitude, and food availability during its exploitation. Most *in vivo* studies on various wound models suggest the beneficial roles of propolis in experimental wound healing, which has also been confirmed in clinical trial studies. However, there is a lack of information concerning the dosage, side effects, and clinical effectiveness of propolis on wounds [2].

This study is divided into two parts: the first is an *in vitro* study aiming to prepare an ointment based on ethanolic extract of Algerian propolis, while the *in vivo* part aims to evaluate the effect of propolis on the healing of second–intention skin wounds in rabbits.

MATERIALS AND METHODS

Bacterial sensitivity tests and the *in vivo* studies were conducted at the Laboratory of Hygiene and Animal Pathology, University of Tiaret, Algeria (Code W0610800), in March 2024.

In vitro Study

Origin and chemical composition of propolis

Two samples of Algerian propolis, collected from distinct floral and geographical origins by renowned beekeepers, were analyzed [7]:

- Sample P1: Harvested from the Tipaza region, Algeria (Altitude: 36°37'4.36" N, Longitude: 2°27'4.36" E). It contained 35.3 mg of total polyphenols (expressed as Gallic Acid per gram of propolis) and 16 mg of total flavonoids (expressed as Quercetin per gram of propolis).

- Sample P2: Collected from the Souk Ahras region (Hnanncha), Algeria (Altitude: 36°15'40.69" N, Longitude: 7°47'16.29" E). It had 49.7 mg of total polyphenols (Gallic Acid per gram of propolis) and 23.3 mg of total flavonoids (Quercetin per gram of propolis).

Propolis extraction

Propolis cannot be used in its raw form and requires purification through solvent extraction to remove inert materials while preserving the polyphenolic fraction essential for experiments. The extraction was conducted in the Biochemistry Laboratory of the Faculty of Natural and Life Sciences, Tiaret, using a traditional method. A total of 20 g of raw propolis, weighed with an analytical balance (Kern – max 120 mg –Germany) was mixed with 200 mL of 70% ethanol in light–protected flasks and periodically stirred with a magnetic stirrer in the dark, following the protocol previous [8, 9, 10]. The ethanolic solution was filtered using Whatman filter paper No. 1. The filtrate was placed in petri dishes, and the ethanol was evaporated in an oven (Heraeus, Germany) at 45°C. The purified propolis extract was scraped off and stored in the dark at 4°C (Condor, Algeria) for further use.

Bacterial sensitivity testing

Preparation of the bacterial inoculum

Müller–Hinton agar, liquefied using a microwave (Samsung GE86V, Malaysia), was poured into petri dishes and allowed to solidify. The bacterial load was adjusted using a spectrophotometer (Hitachi, Japan) to an optical density of 0.08 – 0.13, corresponding to 0.5 McFarland standards. Petri dishes were inoculated with 1 mL of *Staphylococcus aureus* (ATTC 25923), provided by the Microbiology Laboratory of the Faculty of Sciences, Tiaret, and incubated (Memmert, Germany) for 15 min.

Antimicrobial activity testing using the disk diffusion method

The antimicrobial activity of ethanolic extract of Algerian propolis (EEPA1, EEPA2) was evaluated at the University of Tiaret, using the disk diffusion method on Müller–Hinton agar. Sterilized 6 mm disks were soaked in 100 mg·mL^{-1} propolis solutions and placed on inoculated agar plates alongside a sulfadiazine disk (positive control) (FIG. 1A and 1B). After 2 hours (h) of diffusion at room temperature and 24 h of incubation at 37°C, inhibition zones were measured with calipers to assess efficacy [7, 11].

Preparation of the galenic form

- **Preparation of the oily phase**: The oily phase is prepared using 40 g of pure petroleum jelly as an excipient, which requires gentle heating to melt.

- **Dispersion of the active ingredient**: The active ingredient, EEPA, at a concentration of 100 mg·mL^{-1} and typically present in small

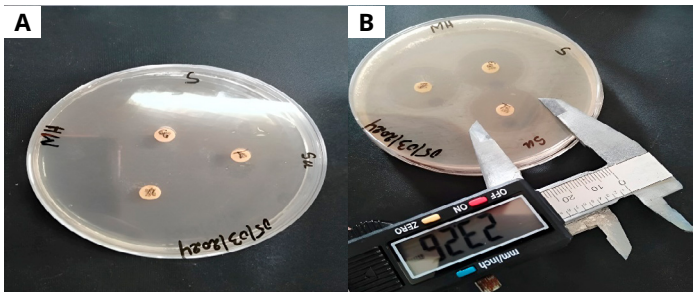

FIGURE 1. A: Deposits of the three disks (sulfadiazine, propolis extracts EEPA1/ EEPA2 and ethanol control). B: Measurement of the inhibition zone (mm)

amounts (2 g) in the formulation, is uniformly dispersed under dark conditions to optimize the product's yield and efficacy. This method is adapted from Sene *et al.* [12], with slight modifications (FIG. 2). In this study, we specifically work with P2, a propolis variety rich in polyphenols and flavonoids, characterized by its moderate bacterial sensitivity, which was confirmed through *in vitro* testing prior to its use in the formulation.

FIGURE 2. Final Product: Ointment based on EEPA (Ethanolic Extract of Algerian Propolis)

In vivo Study

Experimental Description

Nine adult male New Zealand rabbits, aged 6 months to 1 year and weighing (Microlife, France) approximately 3 kg, were used in this study. The rabbits were housed individually under controlled conditions at 25°C, with unlimited access to water and a daily diet of 120 g consisting of alfalfa meal (*Medicago sativa*), corn (*Zea mays*), and soybean (*Glycine max)*.

The rabbits were divided into three groups (three rabbits per group):

1. **Propolis Group**: Treated topically with EEPA on a surgically induced 16 cm² wound.
2. **Silver Sulfadiazine Group**: Treated with silver sulfadiazine following the same surgical procedure.
3. **Control Group**: Received no treatment.

Surgical site preparation

The operative site was carefully shaved, then meticulously cleaned with water and soap, followed by a detergent to eliminate both transient and resident microbial flora.

Anesthetic protocol

Anesthesia began with premedication using a dual injection of Acepromazine (0.75 mg·kg^{-1}, IM) providing tranquilization and Xylazine (2.5 mg·kg^{-1}, SC) ensuring perioperative and postoperative analgesia. Narcosis was achieved with ketamine administered intramuscularly at 35 mg·kg^{-1} [13, 14].

Surgical Procedure

The dorsal skin of the animal was shaved and disinfected. A 4 cm × 4 cm square wound was marked using a ruler and marker (FIG. 3A). After positioning the surgical drape, a full–thickness skin excision was performed. This involved four precise and deep incisions along the pre–marked lines using a No. 11 scalpel blade. The skin flap was then removed by dissection with Metzenbaum scissors and forceps (FIGS. 3B, 3C and 3D).

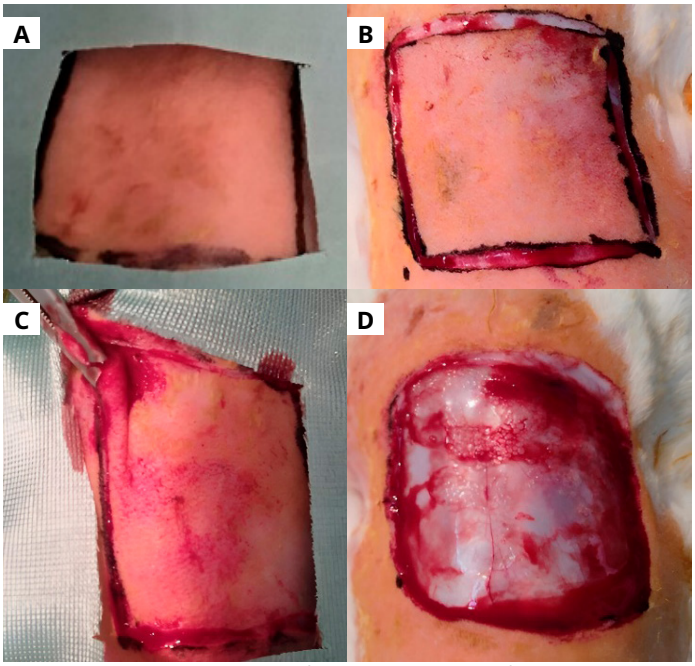

FIGURE 3. A: Skin marking of 16 cm². B: Skin incision 16 cm². C: Dissection of the skin flap. D: Surgical wound with a 16 cm² loss of tissue

For the treatment of wounds in the first group, a thin layer of EEPA–based ointment was applied. In the second group, a 2 mm–thick layer of 1% silver Sulfadiazine cream was used. In both treated groups, a sterile dry dressing was applied approximately 10 min after the ointment application. This approach aimed to prevent the dressing from absorbing the entire amount of the product whether EEPA–based ointment or silver sulfadiazine while maintaining a favorable environment for the healing process. The control group received no treatment.

Clinical follow–up parameters

The wounds were protected from external contamination by covering them with a dry, sterile dressing, which was changed every 24 h. A macroscopic evaluation of the wound, along with photographs, was taken at each dressing change until complete healing was achieved. Additionally, daily rectal temperature measurements (Rossmax, China) were taken for up to 15 d.

Macroscopic wound evaluation parameters

A macroscopic evaluation of the wound was performed over a five–week period, from d 1 to d 36. In accordance with the guidelines of Khan and Peh [15], this evaluation involved assigning scores from 0 to 3 based on several criteria, including the presence of exudates, color changes, hydration status, and the odor of the wound.

a. Wound evaluation

The wound evaluation was based on criteria for optimal healing, including the absence of exudates, odor, color changes, and dryness. A score of 3 indicates excellent healing, while a score of 0 reflects poor healing. Intermediate scores of 1 and 2 correspond to weak and moderate healing, respectively.

b. Dressing evaluation

This evaluation assessed the flexibility, moisture retention, and ease of removal of the dressing using a scale from 0 to 3. An optimal dressing that is flexible, can be removed without damaging newly formed tissue, and prevents liquid accumulation receives the highest score of 3.

c. Extent of wound contraction

In line with the recommendations of Wendelken *et al.* [16], the extent of wound contraction was evaluated by calculating its percentage. To measure the surface area of the wounds, the outline of the lesions was traced on transparent acetate paper with a fine pencil and then transferred to graph paper.

The percentage of wound contraction over time was calculated using the following formula, as described by Subalakshmi *et al.* [17]:

$$Wound\ \ shrinkage\ \ percentage\ \ = \left(\frac{Initial\ \ wound\ \ size\ \ -\ \ Wound\ \ size\ \ on\ \ a\ \ specific\ \ date}{Initial\ \ wound\ \ size}\right) \times 100$$

To measure the wound area, the perimeter of the lesion was traced on transparent acetate paper with a fine pencil and transferred to graph paper [16].

To improve the accuracy of wound surface area calculations and avoid errors related to the intersection of tracing lines, a more precise method based on dividing squares into halves, thirds, and quarters [16]. To achieve this, the circumferences initially outlined were digitized using computer–aided design (CAD) software, specifically AUTOCAD 2016, in accordance with the methods described by Amorim *et al.* [18], Barral *et al.* [19]. These techniques were applied as illustrated in FIG. 4.

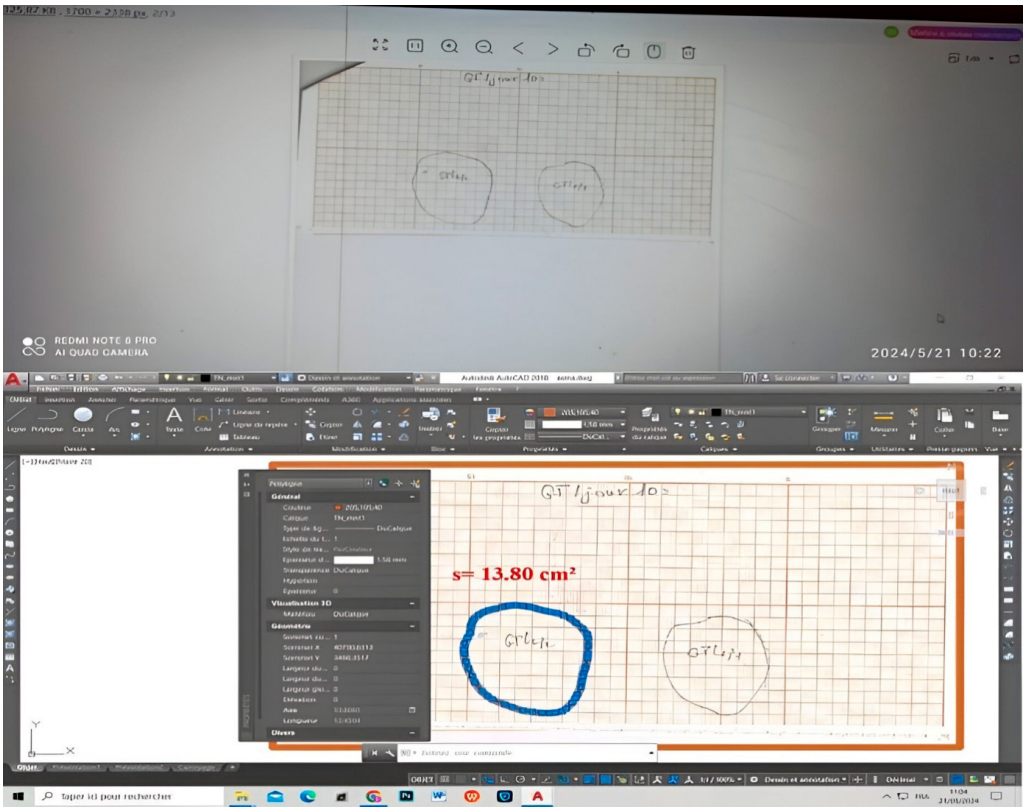

FIGURE 4. Steps for calculating wound surface areas

Statistical analysis and data processing

For the temperature variable, mean values and standard deviations were calculated for the three groups (propolis, sulfadiazine, and control). A statistical analysis was performed using IBM SPSS® version 27, employing a one–way ANOVA to evaluate the impact of temperature on the treatment outcomes.

RESULTS AND DISCUSSION

Wound healing by secondary intention occurs when the edges of the wound are not brought together, allowing the wound to heal from the base upwards. This process is generally longer and is subject to an increased risk of infection and scarring. Propolis, a resinous substance collected by bees, has shown antimicrobial, anti–inflammatory, and healing properties that can accelerate healing in secondary intention wounds. It promotes the formation of new tissue while protecting against infections, which can improve healing outcomes and reduce scarring.

In vitro Part

Antimicrobial Activity of Propolis

Staphylococcus aureus, a common wound pathogen resistant to multiple antibiotics [20], makes it a suitable model for evaluating the efficacy of natural antimicrobial agents like propolis.

The results (TABLE I and FIGS. 5A and 5B) show that the inhibition zones of propolis against *Staphylococcus aureus* vary slightly between samples, with average values of 9.5 ± 1.04 mm for sample P1 and 11.8 mm for sample P2. These results indicate moderate bacterial sensitivity, with sample P1 being slightly less effective than P2. No antibacterial activity was observed for the controls using ethanol or petroleum jelly.

These findings are consistent with the work of Bonvehi and Gutiérrez [21], who found inhibition zones of 10 to 16 mm against *S. aureus* with propolis collected from various regions in Northern Spain. Similarly, Gonsales *et al.* [22] observed inhibition zones ranging from 8 to 13 mm against *S. aureus* with propolis from different origins. This antimicrobial activity is attributed to the polyphenols in propolis, which disrupt bacterial transpeptidation, thus enhancing the effectiveness of β–lactams against staphylococci, [18, 23, 24]. Propolis combats pathogens by blocking their replication, preventing cell invasion through enzyme inhibition and barrier formation, and disrupting their energy metabolism by targeting key organelles [25]. These results highlight the potential of propolis as a complementary therapeutic agent in the treatment of skin infections, particularly in the context of rising bacterial resistance.

TABLE I
Inhibition Zones Of Propolis And Sulfadiazine Against *Staphylococcus aureus*

Strain *Staphylococcus aureus*				
Inhibition zone of Propolis (mm)		Inhibition zone of Sulfadiazine (mm)	Inhibition zone in the control group (mm) (Ethanol)	Inhibition zone of petroleum Jelly (mm)
P1	P2	22.1 ± 1.54	00	00
9.5 ± 1.04	11.8			

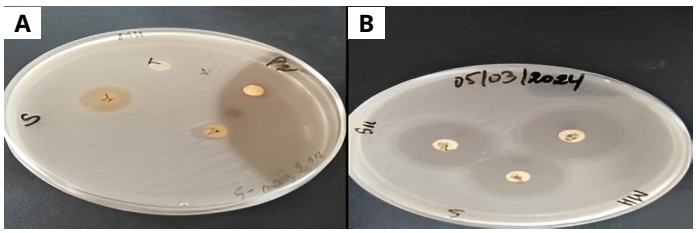

FIGURE 5. A: Results of the antibacterial effect of Propolis against *Staphylococcus aureus*. B: Results of the antibacterial effect of Sulfadiazine against *S. aureus*

In vivo Part

Body temperature monitoring

These results suggest that the rabbits in all three groups maintained normal and stable rectal temperatures (Rossmax, China) throughout the 15 d period. The average temperatures recorded were 38.00 ± 0.68°C for the Propolis group, 38.19 ± 1.01°C for the silver sulfadiazine group, and 37.91 ± 0.59°C for the control group. No statistically significant differences were observed between the groups (P=0.345), indicating that the treatments had no appreciable effect on the rabbits core body temperatures (Table II).

TABLE II
Body temperature for each group

Body Temperature	PG	SDG	CG
Mean ± SD	38.00 ±0.66°C	38.19 ±1,01°C	37.91 ± 0,58°C

PG: Propolis Group, SDG: Silver Sulfadiazine Group, CG: Control Group

Rectal temperature in rabbits, which has long been neglected due to its potential stress–inducing effects, is now recognized as an important prognostic indicator, particularly in rabbits under anesthesia. The normal rectal temperature in rabbits ranges from 38 to 39.9°C, with hypothermia defined as a temperature below 37.9°C [26]. According to Clark–Price [27], a complex regulatory system allows rabbits to maintain a stable body temperature even in the face of environmental variations. It is essential to maintain this temperature for optimal metabolism, which results from a balance between heat production (thermogenesis) and heat loss (thermolysis).

No significant difference was observed between the three groups (TABLE II). The treatments administered, as well as the anesthesia protocol, did not have any harmful effect on body temperature. However, a slight decrease was recorded in the control group (37.91 ± 0.59°C), which appears to be attributable to the large wound surface area (16 cm²).

Macroscopic parameters for wound assessment:

This evaluation involves assigning scores ranging from 0 to 3 based on several criteria, including the presence of exudates, color changes, hydration status, wound odor, and measurement of wound size.

Wound contraction rate

The wound contraction rate is assessed by measuring the reduction in wound size over time, reflecting the healing progress and the effectiveness of the treatment. The results in TABLE III and FIG. 11 indicate that the group treated with the propolis–based ointment achieved complete healing of a 16 cm² wound within 16 to 30 d. In the group treated with sulfadiazine, wound healing required 24 to 36 d, but it was not fully completed, with a contraction rate of 95.06%. Unfortunately, one rabbit in this group died on the second d. For the control group, wound contraction remained incomplete even after 36 d, with the healing process showing minimal progress. Additionally, one rabbit in this group also died in the early d of the study.

TABLE III
Speed wound contraction / Contraction rate (%)

Animals	Wound contraction rate / Contraction percentage (%). Initial Size: 16 cm²					
	PG		SDG		CG	
	SC	WCR	SC	WCR	SC	WCR
R1	16 days	100%	24 days	100%	36 days	99.37%
R2	24 days	100%	36 days	95.06%	36 days	93.75%
R3	30 days	100%	Deceased	/	Deceased	/

SP: Speed contraction, WCR: Wound contraction rate, SDG: Silver sulfadiazine, PG: Propolis group, CG: Control group, R: Rabbit

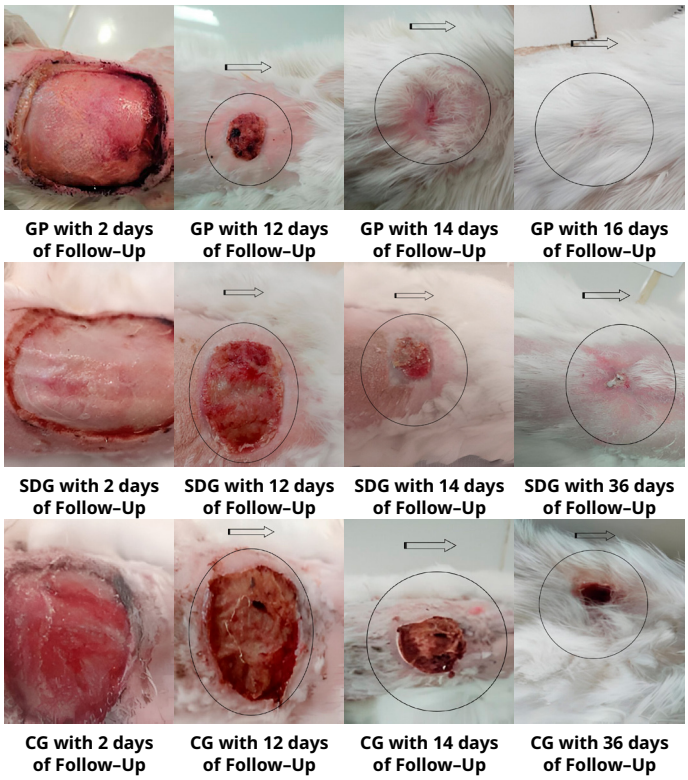

FIGURE 11. Wound healing dynamics in different groups over time

Wound Evaluation

Based on the data from TABLE IV and FIG. 11 , the wound healing assessment, which is based on optimal healing criteria (absence of exudates, odor, color change, and dryness), showed a healing score ranging from 2.27 ± 0.59 to 2.80 ± 0.46 for the group treated with propolis. This indicates moderate to excellent healing, highlighting the effectiveness of propolis in promoting wound healing compared to the other groups TABLE IV.

Wound healing involves contraction, which leads to wound closure. Thus, clinical characteristics and contraction measurements become reliable indicators for macroscopically assessing wound healing [28].

The results in TABLE III and FIG. 11, show that the group treated with a propolis–based ointment completed wound healing of a 16 cm² wound in 16 to 30 d. In comparison, wounds in the sulfadiazine–treated group took approximately 24 to 36 d to heal, without achieving full closure, with a contraction rate of 95.06%. Unfortunately, one rabbit in this group died on the second day. The control group did not show complete contraction even after 36 d. According to El–Sakhawy *et al.* [29], propolis cream enhances skin healing and reduces inflammation more effectively than silver sulfadiazine.

The findings of this study are consistent with those of Da Rosa *et al.* [30], who emphasized the importance of contraction in the healing of full–thickness wounds. Natural products promote healing through antimicrobial, anti–inflammatory, and antioxidant mechanisms, as well as by stimulating collagen production, cell proliferation, and angiogenic effects. The application of propolis extract in wound treatment reduces healing time and enhances both wound contraction and tissue repair, primarily due to its antimicrobial and anti–inflammatory properties [31]. When incorporated at 1% into polyurethane–hyaluronic acid dressings, ethanolic propolis extract significantly accelerates the healing process [29].

Velho *et al.* [32] reported comparable results using Egyptian, Brazilian red, and Iraqi propolis, with more than 90% wound regeneration observed after 14 d. Interestingly, even among studies using propolis from the same geographical origin, outcomes varied considerably. Some studies achieved complete wound regeneration (100%) by day 12 of treatment, while others reported only 30% regeneration after 14 d. Da Rosa *et al.* [30] evaluated the effectiveness of a 30% propolis ointment in the healing of various types of ulcers (venous, pressure, and diabetic). In their study, the average healing time was 45 d, with 20% of patients achieving complete wound closure. In contrast, in the present study, using a 10% concentration of propolis, complete wound healing was achieved within just 14 d. According to Yang *et al.* [25], silver sulfadiazine is commonly used to prevent or treat wound colonization, including infections caused by antibiotic–resistant bacteria. However, *in vitro* studies using acute wound models in rats have shown that topical antibacterial agents such as silver sulfadiazine and mafenide acetate can be cytotoxic to fibroblasts, which may explain the delayed healing observed up to 36 d.

In this study, the wounds of animals treated with propolis healed faster than those of animals treated with sulfadiazine and those in the control group. The delayed healing in the other groups may be due to slower reepithelialization and less significant tissue contraction.

TABLE IV
Healing scores for each group

Groups	Mean ± SD
R1PG	2,80 ± 0,46
R2PG	2,75 ± 0,5
R3PG	2,63 ± 0,63
R1SDG	2,63 ± 0,54
R2SDG	2,61 ± 0,54
R1CG	2,31 ± 0,71
R2CG	2,27 ± 0,59

RPG: Rabbit (1,2,3) Propolis group. RSDG: Rabbit (1,2,3) Silver Sulfadiazine group. RCG: Rabbit (1,2,3) Control group

The healing evaluation reflects the following criteria:

- ***Exudate Evaluation:*** Exudates were observed in the silver sulfadiazine and propolis groups only during the first week, and only on the first day of follow–up for the control group.
- ***Odor Evaluation:*** The wounds in the rabbits treated with propolis and sulfadiazine showed no perceptible odor during the study period, while the control group presented an odor from the first day.
- ***Color Evaluation:*** No significant differences were noted between the groups, except on d 2, 3, and 8 for the silver sulfadiazine group, and 2, 3, and 5 for the propolis group, where a transient change in wound color was observed.
- ***Hydration Evaluation:*** The hydration of the wounds in the rabbits showed low initial hydration, followed by a complete absence of hydration on d 6, 9, and 10 for the propolis group and from d 8 to 17 for the sulfadiazine group. In contrast, the control group's wounds began to dry out from d 5 to 20.

The results in TABLE IV and FIG. 11 show that the propolis–treated group achieved healing scores ranging from 2.63 ± 0.63 to 2.80 ± 0.46, classified as moderate to excellent. These results highlight the effectiveness of propolis in promoting wound healing compared to the other groups.

Exudate was observed only during the first week for the groups treated with silver sulfadiazine and propolis, and only on the first day for the control group. These observations are in line with the findings of Romanelli [32], Xu *et al*. [33], who emphasized the importance of a balanced and moist environment to promote wound healing, while also pointing out the risks of complications due to excessive exudate.

Xu *et al*. [33] Stressed the importance of a moist environment for wound healing, stimulating cell growth and collagen proliferation. In agreement, Boudra and Benbelkacem [34] found that applying propolis to infected wounds in rabbits significantly reduced unpleasant odors, attributed to its antimicrobial action limiting bacterial growth.

Da Rosa *et al.* [30] demonstrated the antibiotic effect of propolis through the analysis of samples collected from wound beds, revealing that propolis effectively reduced local infection and stimulated granulation tissue formation. This observation explains the progression of wound healing in the propolis–treated group without the development of necrosis.

Kim and You [35] suggest that the use of propolis improves the quality of damaged hair. A significant difference in fur regrowth was observed between the treatment groups. In the propolis–treated group, hair regrowth occurred in a shorter time compared to the sulfadiazine and control groups, indicating quicker skin recovery and better overall wound health. Furthermore, after complete healing, the wounds in the propolis group had a more aesthetically pleasing appearance. The wound edges appeared more regular, and the skin texture around the healing area was smoother, suggesting better skin healing quality. This observation highlights that propolis not only accelerates the healing process but also improves the aesthetic appearance of healed wounds

Dressing Evaluation

Dressing Evaluation Scores, including their flexibility, water retention, and ease of removal, are essential for preserving granulation tissues. The results of the evaluation scores for these parameters over time are summarized in TABLE V.

The dressing evaluation scores are highest in the propolis group (2.5 ± 0.51), followed by the sulfadiazine group (RSDG) (2.25 ± 0.44), and the control group (1.5 ± 0.81). This suggests that rabbits treated with propolis (RPG) had a better progression of their dressing compared to those treated with sulfadiazine or the control group rabbits (RCG).

TABLE V
Dressing evaluation scores for each group

Groups	Mean ± SD
R1PG	2,50 ± 0,51
R2PG	2,50 ± 0,51
R3PG	2,50 ± 0,51
R1SDG	2,25 ± 0,44
R2SDG	2,25 ± 0,44
R1CG	1,50 ± 0,81
R2CG	1,50 ± 0,81

RPG: Rabbit (1,2,3) Propolis group. RSDG: Rabbit (1,2,3) Silver Sulfadiazine group. RCG: Rabbit (1,2,3) Control group

According to Da Rosa *et al.* [30], the long–standing controversy between dry and moist dressings has evolved in favor of current occlusive techniques that aim to maintain a moist environment, now widely recognized as beneficial for wound healing. Modern dressings are often enriched with active substances such as silver sulfadiazine (with antibacterial properties), enzymes like collagenase (for debridement of devitalized tissue), and essential fatty acids (used in the prevention and treatment of pressure ulcers). In the present study, the behavior of dressings varied

among groups, providing valuable insights into the wound healing process. Dressings in the control group remained almost dry throughout the evaluation period, indicating minimal exudation and a likely slow or inactive healing process. In contrast, wounds treated with propolis exhibited fluid accumulation during the first eight days, which gradually resolved by d 30. This transient moisture retention suggests an active early inflammatory phase, likely stimulated by the bioactive compounds in propolis, thereby promoting tissue regeneration [25, 30]. For wounds treated with silver sulfadiazine, moderate fluid retention was observed on d 5, 6, and 7, followed by dryness, which indicates a milder or delayed inflammatory response, probably related to the antiseptic effect of the molecule [25]. Moreover, a notable difference was observed in the removal of dressings: it was difficult in the control group, whereas it was easy and atraumatic in animals treated with propolis and sulfadiazine. These findings are supported by a previous study demonstrating that the use of a propolis–based hydrogel in rats promoted rapid wound healing. The dressings were positively evaluated for their flexibility and ease of removal, as they facilitated debridement, maintained a moist environment, and protected the wound from infection. These observations explain the easier removal of dressings in subjects treated with propolis and silver sulfadiazine [36].

CONCLUSION

In vitro studies revealed moderate bacterial sensitivity of propolis against *Staphylococcus aureus*, confirming its antimicrobial potential. *In vivo*, rabbits treated with a propolis–based ointment demonstrated accelerated wound healing, improved aesthetic appearance of scars, and better dressing management, including enhanced flexibility and ease of removal. Additionally, a significantly faster hair regrowth was observed in the treated groups. These findings highlight the therapeutic potential of propolis as a promising alternative in wound care management.

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Conflict of interest

The authors declare no conflicts of interest related to this report

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