Skin wound healing using topical treatment with própolis

https://doi.org/10.56238/sevened2024.010-011

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ABSTRACT

This chapter presents the concepts of skin and the mechanisms of wound healing of this organ, along with concepts of some pathways and cellular dynamics in the processes with topical propolis treatment in different in vivo models.

Keywords: Skin wound, Treatment, Própolis.

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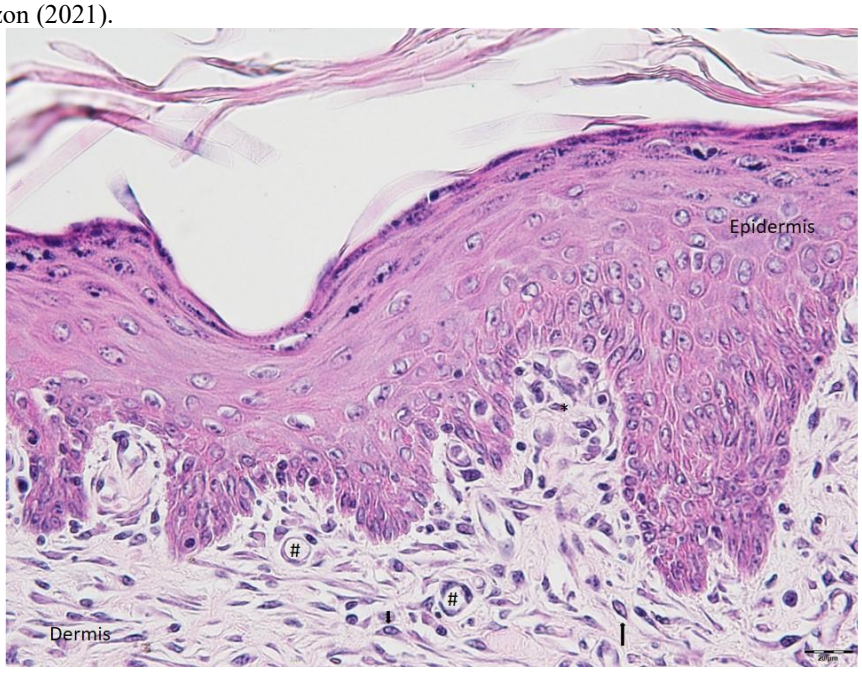
INTRODUCTION

The skin is a fundamental organ of the body and essential to its integrity. It is also the primary mechanical barrier, isolating the external environment, maintaining hydration and electrolyte homeostasis, regulating body temperature, and facilitating Vitamin D synthesis (Kolimi et al., 2022; Proksch et al., 2008).

The skin comprises three layers: the epidermis, dermis, and hypodermis (Fig. 1). The epidermal layer consists of stratified squamous epithelium with keratinization and continuous renewal. The primary cell type in the epidermis is the keratinocyte, which undergoes morphological changes transitioning from cuboidal to squamous shape modified into layers: basal (also known as germinative), spinous, granular, lucid, and corneal layers (Gilchrest, 1983; Khavkin & Ellis, 2011a; Raja, 2007). Keratinocytes is major cell that form the epidermal barrier and play a crucial role in re-epithelialization. They can cover wound surfaces to regenerate an epithelial barrier with the external environment, constituting the body's first physical defense barrier. The keratinocytes secrete multiple cytokines to stimulate re-epithelialization, angiogenesis, the production of connective tissue matrix, and innate immunity. After injury and microbial invasion, keratinocytes release various cytokines, chemokines, and antimicrobial peptides (AMPs) that activate immune cells and directly eliminate pathogens (Fang & Lan, 2023; Piipponen et al., 2020).

Figure 1 - Photomicrograph of rat skin showed with epidermis and dermis. The epidermis including cell layers and many keratinocytes, the dermis can observe the papillary dermis (*); fibroblasts (arrows); and blood vessels (#). Courtesy by Gushiken & Pellizzon (2021).



Melanocytes, Langerhans, and Meckel cells are other crucial parts of the epidermal layer. Melanocytes found between the basal and spinous layers of the epidermis that secretion melanin by tyrosine via tyrosine kinase and stored in melanosomes are essential for UV light protection.

(Gilchrest et al., 1979; Khavkin and Ellis, 2011b). Langerhans cells are antigen-presenting cells originating in the bone marrow and observed in the spinous layer of the epidermis. These cells have extensive cytoplasmic branching, increasing the surface area for contact with antigens. During aging, there may be a decline in the number and functionality of these cells (Pilkington et al., 2018). Merkel cells are mechanoreceptors located in the basal layer of the epidermis and have embryonic origins in the neural crest. In the epidermis, they interact with adjacent keratinocytes via desmosomes and contact afferent myelinated nerve fibers (Winkelmann, 1977).

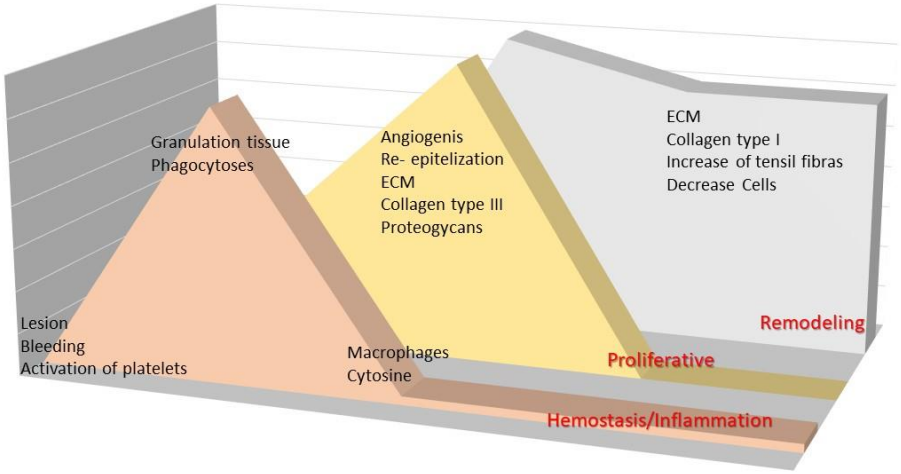
The second layer of the skin is the dermis, soft, irregular, moderately dense connective tissue, and there are two distinct regions: the papillary dermis (soft connective tissue) and the reticular dermis (deeper, moderately dense connective tissue). The papillary dermis, connective tissue cells, fibroblasts, collagen fibers, blood vessels, and many nerve endings can be observed (Losquadro, 2017). In the dermis, we can find the majority of sebaceous and sweat glands and the base of the hair follicles (Jacobsen et al., 1985; Kanitakis, 2002; Khavkin & Ellis, 2011b; Plewig & Kligman, 1978; Zouboulis, 2004).

The hypodermis is the deepest layer of the skin and where the most significant accumulation of unilocular adipose tissue, the essential function for the secretion of vitamin D (Fjeldborg et al., 2014; Khavkin & Ellis, 2011b; Lee & Tontonoz, 2014; Nguyen & Soulika, 2019; Stienstra et al., 2014; Wong et al., 2016).

SKIN WOUND HEALING

The tissue repair process begins with the epidermis, and the dermis is disrupted; this injury is highly concerning clinically because it involves rupturing the body's primary protective barrier. The skin lesions can be acute or chronic; the second one increases the complexity of wound healing, which increases the complexity of repair (Moliterni et al., 2021). Chronic lesions are significantly expensive in healthcare systems (Sen, 2021). The tissue repair process is highly complex and interdependent, with overlapping biological activities (Gantwerker & Hom, 2011) (Fig. 2).

Figure 2: Scheme showing the three main phases of tissue repair, modified from Gantwerker & Hom (Modified by Pellizzon based on Gantwerker and Hom, 2011).



Hemostasis/Inflammation Phase: The hemostasis/inflammation phase begins seconds after the skin structures are disrupted and activates the coagulation process to stop bleeding. Platelets play a crucial role in coagulation, and a series of multiple cytokines, hormones, and chemokines are released and activated by endothelial receptors and dermal cells. Within minutes, there is an influx of inflammatory cells, predominantly neutrophils, and macrophages, essential for the subsequent phases of healing. There is an increase in the release of cytokines and growth factors such as Interleukin 1 (IL-1β), Tumor Necrosis Factor-alpha (TNF-α), Fibroblast Growth Factor (FGF), and Platelet-Derived Growth Factor (PDGF) (Gantwerker & Hom, 2011; Gushiken et al., 2021; Rodrigues et al., 2019; Wang et al., 2004). Due to the influx of neutrophils, macrophages, and lymphocytes to the injury site, the inflammatory phase begins, with intense activity of phagocytic cells to eliminate debris cells and bacteria. This phase involves an increase in pro-inflammatory cytokines such as IL-1β, TNF-α, and Interleukins 6 and 8 (IL-6 and IL-8), crucial for combating infection (Larouche et al., 2018; Robson, 2003). There is also an increase in growth factors, such as Vascular Endothelial Growth Factor (VEGF) and Insulin-like Growth Factor 1 (IGF-1), promoting proliferation of dermal and epidermal cells (fibroblasts, endothelial cells, and keratinocytes) (Gushiken et al., 2021; Larouche et al., 2018; Reinke & Sorg, 2012; Rodrigues et al., 2019).

Proliferative Phase: In the proliferative phase, injured cells continue to secrete growth factors such as FGF/VEGF/EGF-epidermal/TGF-β, promoting migration and proliferation of cells, especially in the dermal region, where fibroblasts, macrophages, endothelial cells, and extracellular matrix synthesis are present (Bennett et al., 2003). Fibroblasts synthesize the extracellular matrix,

especially collagen III, proteoglycans, and fibronectin, which are crucial for the cellular environment, promoting migration of other cell types (Rodrigues et al., 2019; Singer & Clark, 1999).

Remodeling Phase: The remodeling phase involves complete skin repair and naturally depends on the previous phases. There is a decrease in granulation tissue and replacement by synthesized extracellular matrix and apoptosis of cells that migrated to the area. The increase of TGF-β1 promotes fibroblast differentiation into myofibroblasts that promote wound contraction and closure. Matrix Metalloproteinases (MMPs) degrade the previously synthesized matrix, promoting complete reorganization of collagen type I, elastin, and other matrix components. Thus, the biological events of tissue repair after any skin injury are completed, resulting in the reconstruction of all epidermal and dermal elements, leading to increased organ resistance and flexibility (Karppinen et al., 2019; Martins et al., 2013; Reinke & Sorg, 2012; Singer & Clark, 1999).

Topical Products for Skin Wound Healing: The population widely uses topical treatment due to its ease of administration, allowing for self-application and generalizing its possible self-medication (Gushiken et al., 2021). Due to the numerous biological events that occur during tissue repair, it can take years for complete skin recovery. There are only a few products for topical treatment for all phases, so we are looking for a new product that becomes interesting for research and economic importance due to its wide application in human and veterinary medicine.

Among the products used for topical healing treatment, we choose propolis, which has been described for use in the process of skin injury repair, with a worldwide distribution and description of its use for a range of healing properties of propolis. Since 13,000 BC, the Greeks, Romans, and Egyptians understood that propolis could be applied as a medicine in creams and was used in the embalming process of bodies. Propolis is a resinous substance similar to wax produced by bees and used in hives as a sealant for their holes. Due to its high-temperature resistance, this resin liquefies between 60-70°C, and in some types of propolis, we have a much higher melting point, reaching up to 100°C (Kuropatnicki et al., 2013). The chemical composition of propolis varies depending on the region of the plant species used by bees for pollen extraction, resulting in many propolis varieties for study, with different biological actions of this resin. The main components are wax, resin, and phenolic compounds such as phenolic acids, flavonoids, and terpenoids. Terpenoids and flavonoids are the main responsible for the pharmacological activities of propolis (Przybyłek & Karpiński, 2019). Searching for a new product and assessing potential in the healing process depends on understanding the process of second-intention wound healing. The phases are crucial for activating the entire cascade of the healing process and improving the patient's quality of life.

OBJECTIVE

This study aims to identify skin contraction studies using propolis in experimental models to understand the main models used and the state of the art of these studies.

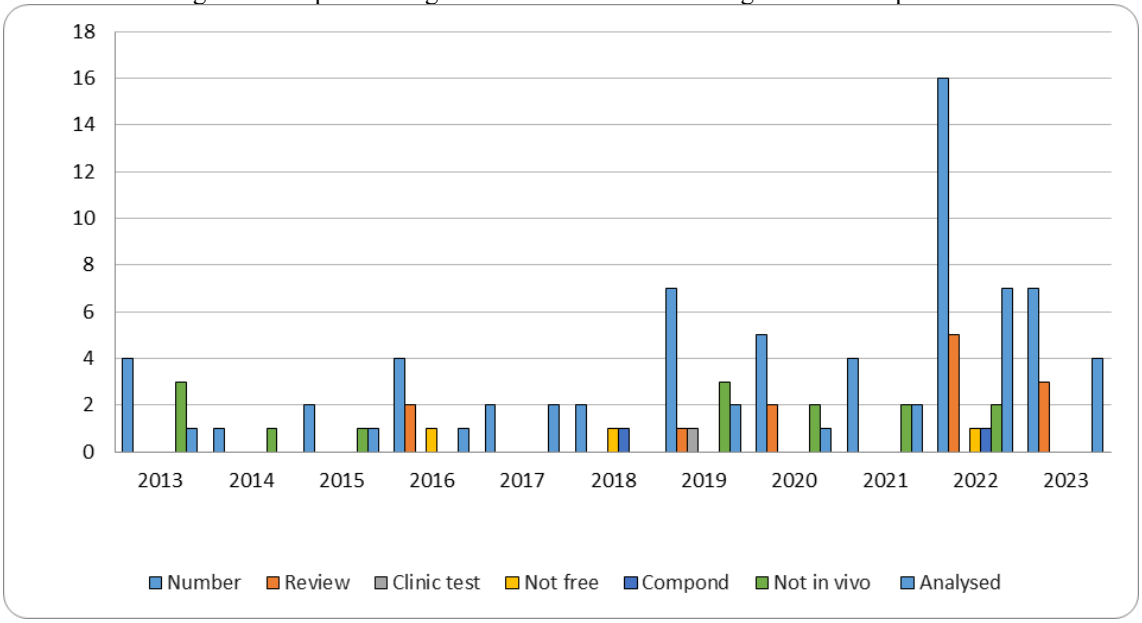
METHODOLOGY

To achieve this, we used the PUBMED search platform (www.pubmed.com) and analyzed articles found in the last 10 years (2013-2023) using the following search terms: "propolis" AND "wound healing" AND "skin" present in the title, abstract, and keywords. Inclusion criteria required articles to include the following topics: in vivo experimentation, topical use of propolis, and English language texts. Exclusion criteria involved articles that did not review articles, case reports, clinical trials, articles not available in unpaid search platforms, articles without in vivo tissue contraction, and articles exclusive to isolated propolis substances. This study was based on the data search methodology of Machado-Velho et al. (2023).

RESULTS

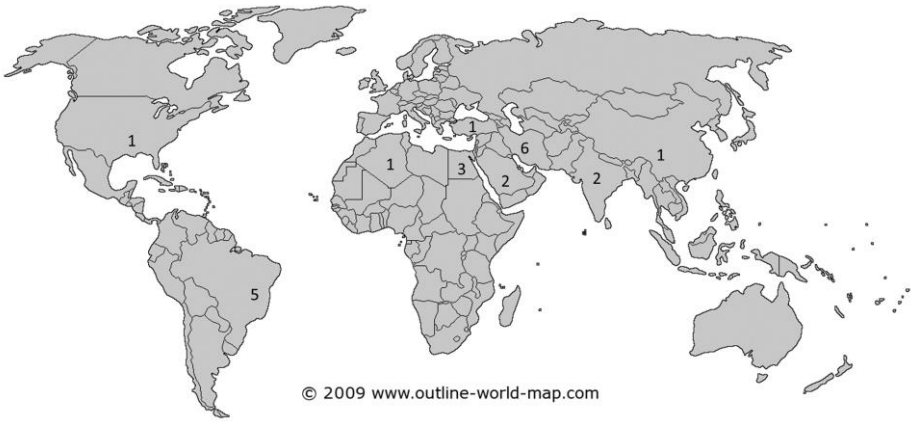
The results of the search on the PUBMED platform yielded 54 articles during the search period, of which the following articles were excluded based on the exclusion criteria: 13 reviews, 1 clinical study, 3 from paid libraries, and 6 without a description of tissue contraction. Within the inclusion criteria, 21 articles met the defined parameters (Fig. 3).

Figure 3: Graph showing the number of articles during the research period.



From the 21 articles that met the parameters of the review search, propolis originated from 9 countries, with the geographic distribution observed in Figure 4.

Figure 4: Biogeographical Location of propolis based on the review process from 2013-2023.



Propolis Formulation: Among the formulations, we observed that most articles used hydroalcoholic preparation in different concentrations, while some authors did not specify the concentration used. The base extraction is hydroalcoholic 70%, one of the most common methods of obtaining active products with more remarkable preservation of active principles. After the initial extraction, several articles promote the development of new formulations, such as nanoparticle associations and non-ionic cream dressings with controlled release of propolis (Table 1).

Table 1: Resume of the article and experimental models with formulations used propolis.

Article	Animal Model	Lesion Model	Country propolis	Topical Formulation
Kapare et al., 2023	Wistar Rat	Excision diameter: 1 cm. Incision Lenght: 3 cm	India	Polyvinyl Alcohol base in hidrogel
Conceição et al., 2022	Wistar Rat	Excision diameter: 2 cm.	Brazil	Hydroalcoholic extract Paste.
Takzaree et al., 2015	Wistar Rat	Excision	Iran	Hydroalcoholic extract
Salrian et al., 2022	Wistar Rat	Excision diameter: 1 cm.	Iran	Propolis 3% and Honey 1,5% in ointment 1,5% honey in ointment
Gupta et al., 2022	Wistar Rat	Excision diameter: 1 cm.	India	Hydroalcoholic extract 2%
Ibrahim et al., 2022	Balb/C mouse	*uninformed*	Saudi Arabia	Collagen hydrolysates (CHDs) plus honey-propolis wax
Corrêa et al., 2017	Swiss mouse	Excision diameter: 8 mm	Brazil	Propolis Red Brasilian PRB- 1:3 (W/V)
Abu-Seida, 2015	Dog	Excision: 3cm	USA	Ointment
Rosseto et al., 2017	SK1mouse with hair	Excision: 3,5 mm 6 lesion for animal	Brazil	Hydroalcoholic extract BPE 50:50 w/w. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC)

Barud et al., 2013	Wistar Rat	Excision:6 mm) 3 by animal	Brazil	Bacterial (BC) membranes (W/W) 1,2/2,4/3,6%
Eskandarinia et al., 2019	Wistar Rat (Female)	Excision: 12mm	Iran	
Eskandarinia et al., 2020	Wistar rat (female)	Excision:11mm	Iran	Dressing
Abdellatif et al., 2021	Spragye –Dawley rat	Excision	Egypt	Nanoemulsion
Diab et al., 2022	Wistar rat	Excision	Egypt	Nanoparticles
Hashemi et al., 2023	Srague Dawley rat	Burn	Iran	Dressing with nanoparticles
Du et al., 2023	Balb-c mouse	Excision : 7 mm	China	Dressing with silk fibroin
Pahlevanneshan et al., 2021	Wstar rat	Excision : 11 mm	Iran	Dressing nanocomposite polyurethane
Elkhateeb et al., 2022	Rabbit (New Zealand)	Excision : 3x3 cm	Egypt	Propolis nananostructured lipid carriers
Segueni et al., 2022	Wistar Rat	Excision. 2 by animal	Argeria	Hydroalcoholic extract
Picolotto et al., 2019	Balb/c mouse	Excision 10mm	Brazil	Dressing
Çelik Yilmaz; Aygin, 2023	Balb/c mouse	Excision	Turkey	Hydroalcoholic extract

Application Forms of Propolis: The application forms varied significantly among the analyzed articles. They ranged from diluted propolis applied directly to the wound to developing dressings with nanoparticles and synergy with other elements. Hydroalcoholic formulations are commonly used by the population and in various propolis products. These formulations are prepared after the extraction of raw propolis into a final solution of 70%, similar to what was described by Aldana-Mejia et al. (Aldana-Mejia et al., 2021). Among the analyzed works like Conceição et al. (Conceição et al., 2022) and Takzarre et al. (Takzaree et al., 2015) used 1% propolis, Gupta et al. used 2% propolis (Gupta et al., 2022), Corrêa et al. (Corrêa et al., 2017) used a ratio of 1:3 (w/v), and Rosseto et al. (Rosseto et al., 2017) used red propolis in a 30:70 ethanol (w/w) solution. Conceição et al. (Conceição et al., 2022) and Eskandarinia et al. (Eskandarinia et al., 2019) used a water-based paste (cornstarch) associated with 1% red propolis, 0.5% and 1% propolis, respectively. Eskandarinia et al. also used an association with hyaluronic acid at two concentrations of propolis (Eskandarinia et al., 2019). As a comparative effect of biological actions, Takzarre et al. used a hydroalcoholic solution of 1% propolis and studied its synergy with honey at 3% propolis (Takzaree et al., 2015). Salrian et al. developed synergistic comparison groups with 3% propolis and 1.5% honey (Salrian et al., 2022). Kapare et al. (Kapare et al., 2023) used a PVA formulation in a hydrogel with 5% propolis, exhibiting suitable viscosity and physical stability for 180 days. Eskandarinia et al. (Eskandarinia et al., 2020) developed a dressing with a 1:10 propolis-ethanol ratio associated with polyurethanes alone or combined with gel, showing good viability of the dressing for both biological action and clinical

application in lesions. Propolis was manipulated to prepare dressings as described by Ibrahim et al. (Ibrahim et al., 2022), who used an association with hydrolyzed collagen or with bacterial membrane and cellulose as described by Barud and Picolotto (Barud et al., 2013; Picolotto et al., 2019), or even with an association with nanoparticles (polycaprolactone and quercetin) as described by Hashemi and colleagues (Hashemi et al., 2023) in a burn model. Du and colleagues used dressings with silk fibers associated with gelatin with 1% propolis (Du et al., 2023). There are descriptions of dressings with polyurethane and nano lignin (Pahlevanneshan et al., 2021) and potentially nanostructured lipid carriers (Elkhateeb et al., 2022).

Antibacterial Model: Several studies presented antibacterial studies of propolis, targeting both gram-positive and gram-negative bacteria. The most commonly used bacteria in the studies were *Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli, Pseudomonas aeruginosa*, and *Acinetobacter baumannii.* Different forms of propolis administration have different effects on different bacterial strains. All analyzed articles that conducted antibacterial activity described inhibitory growth tests under the action of different propolis concentrations (Barud et al., 2013; Diab et al., 2022; Du et al., 2023; Elkhateeb et al., 2022; Eskandarinia et al., 2019; Eskandarinia et al., 2020, 2019; Pahlevanneshan et al., 2021; Segueni et al., 2022).

***In vitro* Model**: Antioxidant activities were evaluated in in vitro models. Fibroblasts (murine L-929) and keratinocytes (HaCAT) were used in cell studies. In this review, authors tested the viability of propolis with proposed application associations over time (Abdellatif et al., 2021; Du et al., 2023; Eskandarinia et al., 2020, 2019; Hashemi et al., 2023; Pahlevanneshan et al., 2021; Rosseto et al., 2017).

***In vivo* Model**: The excision model was defined for the review, with various circular, square, and multiple lesions on the dorsal region among the 21 articles used. As for *in vivo* experimental models, we observed one strain of SK1 mice (hairless), one Swiss mouse, four Balb/C mice, two Sprague Dawley rats, eleven Rattus norvegicus (Wistar) rats, one rabbit, and one dog. In mice, the excision size ranged from 3.5 to 10 mm in diameter. The lesions ranged from 6 mm to 2 cm in diameter in rats. In the article that used a rabbit and a dog, we observed lesions of 9 cm2 and 3 cm in diameter, respectively (Abdellatif et al., 2021; Abu-Seida, 2015; Barud et al., 2013; Çelik et al., 2023; Conceição et al., 2022; Corrêa et al., 2017; Diab et al., 2022; Du et al., 2023; Elkhateeb et al., 2022; Ibrahim et al., 2022; Kapare et al., 2023; Pahlevanneshan et al., 2021; Picolotto et al., 2019; Rosseto et al., 2017; Salrian et al., 2022; Segueni et al., 2022; Takzaree et al., 2015).

Experimental Time: There is a significant difference between the experimental analysis times, ranging from 0 to 20 days in mice, 0 to 30 days in rats, 7 to 21 days in rabbits, and 0 to 28 days in dogs (Abdellatif et al., 2021; Abu-Seida, 2015; Barud et al., 2013; Çelik Yilmaz and Aygin,

DISCUSSION

The skin exhibits broad physiological adaptability and resistance to physical and chemical aggressions. Throughout this review process, a significant disparity was noted among study protocols concerning the activity of propolis in tissue repair of this organ. Tissue repair is a multifaceted process reliant on various elements, with cellular response contingent upon molecular factors and the introduction of materials or new products to interact in this highly orchestrated process for organ repair (Vasalou et al., 2023). Thus, understanding the diverse pathways of propolis action became imperative for analyzing scar formation pathways to develop test protocols for new products based on clinical parameters and applications.

Propolis is a natural resinous mixture produced by honeybees from substances collected from various plant sources (Salatino et al., 2011) and distributed across the Americas, Europe, Asia, and Africa. Possessing diverse chemical characteristics, it induces distinct biological responses and has been described since 13,000 BC (Kuropatnicki et al., 2013). Propolis cannot be utilized before purification, so suitable solvents can be extracted to remove unwanted materials while preserving active components, such as polyphenolic fractions (Miguel & Antunes, 2011). Depending on the extraction method, the biological activities of propolis can be intensified, including antimicrobial, anti-inflammatory, antioxidant, and immunostimulatory properties attributed to its chemical composition, which includes flavonoids, aromatic acids, diterpenic acids, and phenolic compounds (Lofty, 2006; Trusheva et al., 2006). This product has sparked global research interest as an alternative to antibiotics (Miguel & Antunes, 2011) because of the immunoregulatory effect of propolis on the production of factors involved in inflammation, such as cytokines, prostaglandins, chemokines, and others (Hu et al., 2005; sales et al., n.d.). Indeed, studies have demonstrated that propolis can induce immunomodulatory effects in animals, influencing macrophage activation, antibody synthesis, and lymphoid organ weight (Çelik et al., 2023; Eyng et al., 2015; Fischer et al., 2010; ORSI et al., 2000).

Many articles confirm the different activities of propolis for antibacterial, antiviral, antifungal, and antiprotozoal purposes. Many factors influence this activity, involving dosage and propolis extraction methods, which can promote differences in antimicrobial activity (Miguel & Antunes, 2011). One hypothesis regarding the antimicrobial activity of propolis suggests that it acts directly on the bacterial membrane, altering ATP activity and reducing bacterial motility (Przybyłek & Karpiński, 2019). Artepel C, present in different propolis types, especially green propolis, emerges as an essential bacterial agent, with its mechanism of action still unclear (Beserra et al., 2021; Veiga et al., 2017).

Thirty-eight percent of the analyzed studies confirm the effect of different propolis types on reducing bacterial activity. This is significant because, in skin lesions, numerous opportunistic

contaminations can either chronic the lesion or complicate chronic diseases such as diabetic wounds, which, in addition to contamination, alter TNF-Alpha synthesis and keratinocyte activity (Fang & Lan, 2023). Keratinocytes undergo a regulated terminal differentiation process to form the stratum corneum, creating an essential physical barrier. This process promotes epidermal renewal, where keratinocytes organize themselves, increasing the synthesis of intermediate filaments and lipid granules, thus promoting a permeability barrier (Harris-Tryon & Grice, 2022).

In vitro models using cell cultures are frequently employed to advance understanding of underlying mechanisms of in *vivo* cellular behavior. Two-dimensional (2D) cell cultures have been utilized as in *vitro* models to study cellular responses to biophysical signals, including cell differentiation, migration, growth, and mechanics, all impacted by their biochemical and biomechanical microenvironment (Duval et al., 2017; Huh et al., 2011). In 29% of the articles in this review, authors used 2D cultures, highlighting cellular viability and stretch repair, using fibroblasts as target cells (HaCAT and L929). Propolis concentrations ranging from 0.1 to 1000 ug/ml were included for cellular viability, with maximum viability described as up to 1 ug/ml (Abdellatif et al., 2021). One article presented the activity of lesion retraction in vitro, indicating significant activities (Ibrahim et al., 2022). As a future experiment, laboratory experiments could be conducted with a user-defined three-dimensional (3D) model closely mimicking the cellular microenvironment. However, creating such a model faces challenges, including constructing the tissue-tissue interface, controlling spatial-temporal distributions of oxygen and carbon dioxide, nutrients and waste, and personalizing other microenvironmental factors that regulate in *vivo* activities (Huh et al., 2011).

In *vivo* studies in all analyzed articles were conducted using different formats and sizes. The definition of the model becomes fascinating because the process of healing skin lesions is highly complex. In the skin, the lesion can result from various severe or chronic processes, from trauma, pressure, burns, or even diseases such as diabetes, which promote either complete wound repair or loss of skin functionality (Hashemi et al., 2023; Singer & Clark, 1999).

Complete skin repair requires several events and a complex synchronization dependent on various cell types in sequential stages that need to be coordinated at different stages to restore injured tissue, involving interrelated and overlapping mechanisms of cell migration and proliferation, extracellular matrix (ECM) synthesis, growth factors, and cytokines that coordinate the process (Gushiken et al., 2021; Proksch et al., 2008; Rodrigues et al., 2019; Singer & Clark, 1999).

The tissue repair process depends on homeostasis and activation of the inflammatory phase, which can vary from 0-7 days, with the peak response between 3-5 days post-injury (Flynn et al., 2023; Isaac et al., 2010). Platelet activation involves cellular aggregation and activation of serum fibrinogen, paralleled by cytokine release (IL-1β) and growth factors (TNF-α, FGF-β, and PDGF) synthesized by platelets and cells in the region, attracting leukocytes (neutrophils and monocytes) to

the injury site. Neutrophils are essential cells in this phase as they release various pro-inflammatory cytokines, such as IL1-β, TNF-α, IL-6, and IL-8, essential for the debridement of necrotic tissue and phagocytosis of pathogenic antigens. At the same time, there is a migration of inflammatory response cells to the region and release of other growth factors, such as VEGF and IGF-1, activating fibroblasts, keratinocytes, and endothelial cells (Beldon, 2010; Flynn et al., 2023; Gushiken et al., 2021; Isaac et al., 2010). Within the review process, we observed that all analyzed articles included the inflammatory phase within the analysis period; however, in most articles, there was no description of measuring all growth factors, which could increase the understanding of the pathways of action of different propolis in the wound healing and skin repair pathway, hindering the assertiveness of understanding the mechanism of action in tissue repair.

The proliferative phase begins approximately at the end of the inflammatory peak around the fifth-day post-injury, finishing between the 21st and 30th days. It is characterized by fibroplasia, angiogenesis, and reepithelialization. This phase involves intense migration and proliferation of cells and synthesis of granulation tissue with the synthesis of a provisional extracellular matrix, presence of type III collagen, and migration of different cell types such as macrophages, endothelial cells, and fibroblasts, via chemotactic and mitogenic factors due to the release of growth factors (FGF, VEGF, EGF, and TGF-β1) by cells at the injury site (Flynn et al., 2023; Isaac et al., 2010).

The process of cell migration activation to the wounded area occurs centripetally, always initiating from the tissue at the lesion margins. In the case of fibroblasts, their migration is crucial for restructuring the local cellular environment, restoring the extracellular matrix and vascularization essential for complete tissue remodeling (Flynn et al., 2023). Fibroblasts synthesize compounds of the provisional extracellular matrix during the proliferative phase, such as collagen type III, proteoglycans, and fibronectin. PDGF and TGF-β induce fibroblast differentiation into myofibroblasts, expressing α-actin, α-myosin, and desmin, thus possessing contractile and movement capacities within the wounded area. Fibronectin deposition occurs during their migration on the fibrin scaffold. Angiogenesis initiates soon after the injury but is more active during the proliferative phase. Mediators like VEGF and angiopoietins stimulate endothelial cell proliferation, thus promoting vascular system restructuring at the site, crucial for supplying oxygen, and nutrients, and promoting cell migration, proliferation, and extracellular matrix synthesis. Reepithelialization occurs during the proliferative phase, closing the wound and restoring skin barrier function. Keratinocytes are stimulated by growth factors to differentiate and migrate through the extracellular matrix towards the lesion center, promoting reepithelialization (Flynn et al., 2023; Gushiken et al., 2021; Isaac et al., 2010).

The remodeling phase is the last stage of wound healing, starting concurrently with the proliferative phase and lasting up to 2 years. Mechanisms initiated in earlier stages are gradually

deactivated, aiming to normalize epidermal thickness, cellular content, extracellular matrix composition, and blood vessel count as close to pre-injury levels. Granulation tissue decreases, provisional extracellular matrix is replaced, gradual degradation of collagen type III occurs, replaced by collagen type I, and apoptosis of provisional cells that migrated to the wounded area (Flynn et al., 2023; Gushiken et al., 2021; Rodrigues et al., 2019).

Propolis demonstrates high potential in the skin tissue repair process, acting on TGF-beta transduction and reducing levels of MMP, pro-inflammatory cytokines, eicosanoids, and increasing collagen I deposition (Cristiane et al., 2013; da Rosa et al., 2022; Franchin et al., 2016). Due to these characteristics, propolis is highly interesting for developing wound healing products, especially for topical applications. Topical medications play different roles in wound healing stages. In the Brazilian market, products containing neomycin and bacitracin (Nebacetin) are used in the initial phase for their properties in preventing and containing infections, a factor that impairs healing. Panthenol or dexpanthenol-based products act in the proliferative phase, promoting fibroblast proliferation and granulation tissue synthesis and accelerating reepithelialization by promoting keratinocyte migration from the lesion border. Collagenase degrades the extracellular matrix, mainly collagen III synthesized in the previous phase, promoting the migration of endothelial cells and keratinocytes crucial for revascularization and epidermal reconstitution.

Dressings protect the wounded area from external agents and pathogens, reducing the risk of infections and aiding in the reduction of wound contraction time. Developing an effective dressing requires considering the physical characteristics and its biological functional activity suitable for each type of wound. Dressings can have specific purposes, such as antibacterial, debriding, occlusive, absorptive, or adhesive, with hydrocolloids, alginates, and collagen being interesting materials in various presentations like cream, film, gel, and foam.

This review highlights the diversity of study protocols in wound healing research, suggesting the standardization of some analyses for more consistent evaluation. A comprehensive understanding of wound healing processes, the influence of substances like propolis, and the role of different interventions, including topical medications and dressings, can lead to the development of more effective therapies for wound care.

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