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Skin repair after second-intention injury: Understanding some mechanisms

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INTRODUCTION

The skin is the largest organ in the human body and among the functions are homeostasis, protection, sensoriality, thermoregulation and metabolism, in which its integrity is crucial to the health of the body (KOLIMI, et al, 2022; AGARWAL & KRISHNAMURTHY, 2023).

Skin injuries can happen traumatically, surgically, or pathologically, and the mode of repair is determined according to the tissue involved in the injury, both in depth and in tissue loss. Second-intention repair occurs when the lesion is unable to approach the edges (BELDON, 2010).

Skin lesion repair is complex and requires the complex synchronization of several different cell types and involves interrelated and overlapping mechanisms of cell migration and proliferation, extracellular matrix (ECM) synthesis, growth factors, and cytokines that coordinate the process. This process can be divided into three phases: inflammatory, proliferative and remodeling, which occur in a temporal sequence, and overlap (SINGER & CLARK, 1999; PROKSCH et al, 2008; RODRIGUES et al, 2019; GUSHIKEN et al, 2021).

The inflammatory phase begins soon after the injury, a fibrin clot is formed promoting local hemostasis. Cytokines and growth factors are responsible for the recruitment of immune cells, and proliferation of fibroblasts, keratinocytes, and endothelial cells at the site (ISAAC, et al, 2010; GUSHIKEN et al, 2021; FLYNN et al, 2023).

The proliferative phase is characterized by fibroplasia, angiogenesis, and re-epithelialization. Fibroblast growth factors (FGF), vascular endothelial (VEGF), epidermal (EGF), and beta transformer (TGF-β1) are responsible for these events. Fibroblasts synthesize compounds from the provisional extracellular matrix and differentiate into myofibroblasts with contractile capacity and movement through

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the injured area. The proliferation of endothelial cells promotes angiogenesis, restructuring the local vascular system. Keratinocytes migrate through the extracellular matrix towards the center of the lesion, promoting re-epithelialization and thus restructuring barrier function (ISAAC et al., 2010; QING, 2017; GUSHIKEN et al., 2021; FLYNN et al, 2023).

In the remodeling phase, there is a decrease in granulation tissue, replacement of the provisional extracellular matrix, and apoptosis of the provisional cells that have migrated to the injured area (ISAAC et al, 2010; GUSHIKEN et al, 2021; FLYNN et al, 2023).

The prevalence of skin lesions is still very high, which highlights the need for further studies in search of new therapies for this purpose. Phytotherapy can be explored for this purpose, as biodiversity is great (JUVINO et al, 2021).

In this scenario, propolis is inserted, a substance produced by bees, with antibacterial, antifungal, anti-inflammatory, healing properties, etc. The biological effects on the body with its use are related to the chemical profile and composition of each type of propolis. Faveleira propolis comes from the plant *Cnidoscolus Quercifolius*, found in abundance in northeastern Brazil, popularly known for its anti-inflammatory, antiseptic properties, etc., (LUSTOSA et al, 2008; COTTICA et al, 2011; CARDINAULT et al, 2012; ALVES & KUBOTA, 2013; MARCUCCI & ANGELA, 2018; MOURA et al, 2019; SANTOS et al, 2020). Therefore, the objective of this work is to investigate the biological, chemical and microbiological properties of faveleira propolis and its action on skin repair in a second-intention model in an experimental lesion.

MATERIALS AND METHODS

Male *Wistar* rats were kept for the experiment for two periods (3 and 7 days), separated into groups: Physiological (SHAM), Negative Control (untreated wound), Positive Controls treated with the following drugs: 1- Neomycin sulfate 5 mg/g + Zinc bacitracin 250 IU/g; 2- Dexpanthenol 50 mg/g and 3- Collagenase 0.6 IU/g, and Tests, in which the animals were treated with hydroalcoholic solutions of Faveleira propolis from Bahia, Brazil, in the percentages of 0.5%, 1% and 2%. The animals were submitted to a dorsal wound of second intention and treated according to their respective experimental groups. All laboratory practices were carried out in accordance with the ethics committee for the use of animals (CEUA IBB-5550250222).

The study included the analysis of clinical parameters, including the presence of crust, exudate, hemorrhage, clot, granulation tissue and epithelialization, considering a score from 0 to 3, with 0 being no and 3 being very present of the parameter (MARTINS, P.S. et al., 2003), in addition to histopathological analyses with hematoxylin and eosin stains, for the quantification of total cellularity, and Masson's trichomic test for the deposition of collagen fibers in the dermis.

The analyses of the clinical parameters and the retraction of the lesion were performed based on the images collected during the experiment. Histopathological analyses were performed from skin samples from the lesion region collected after euthanasia of the animals. Statistical analyses with parametric data were expressed as mean ± standard error of the mean and the comparison between the groups was performed by ANOVA followed by Tukey's test. Analyses with non-parametric data were expressed as median (maximum and minimum) and performed using the Kruskal-Wallis test, followed by Dunn's test. Values of $p > 0.05$ were considered statistically significant.

RESULTS

This work generated relevant data on the use of faveleira propolis in the healing process. Through macroscopic and microscopic morphological characterization carried out in treatment periods of 3 and 7 days, it was possible to analyze some modifications throughout the healing phases.

The choice of topical drugs used as positive controls was due to their action in each of the healing phases. Drugs containing neomycin and bacitracin are used in the initial phase for their properties to prevent and contain infections (WARD & SAFFLE, 1995). Dexpanthenol and collagenase indicate effectiveness in the process of reducing the distances from the edges in second-intention lesions, due to their important role in the dermis. especially in contractile cells (BERRY et al., 1998; EBNER et al., 2002; McCALLON et al., 2015).

Clinical analysis and observation of lesion retraction are important for macroscopic verification of lesion evolution, providing a detailed clinical analysis and verifying the repair aspects of skin disruption. In addition, microscopic histopathological analyses are also important, as they allow a panoramic view of the components of the dermis in the given periods.

INITIAL INFLAMMATORY PHASE (3 DAYS)

The clinical parameters analyzed did not present statistical differences between the groups. Wound retraction can occur due to the action of contractile cells, such as myofibroblasts, which bind to collagen fibers, pulling them towards themselves and contracting the lesion (ISAAC et al., 2010). The groups treated with faveleira propolis showed retraction of 5% to 20%, unlike the pharmacological control groups, which showed edema at the edge of the lesion area. This result suggests that faveleira propolis can exert an important activity in this phase, influencing the agility of lesion closure. The histopathological microscopic analysis of collagen cells and fibers showed favorable results, with higher values in the groups treated with faveleira propolis, in the marginal region of the lesion, in agreement with the result presented by retraction.

PROLIFERATIVE PHASE (7 DAYS)

In this phase, there is an increase in growth factors that influence cell proliferation and differentiation, resulting, among several events, in the deposition of collagen fibers in the dermis (for review, see RODRIGUES et al., 2019; GUSHIKEN et al., 2021).

At this point in the experiment, similar clinical quality is maintained between the test groups and the pharmacological groups. In our samples, through microscopic analyses of cell quantification and collagen fiber deposition, we observed a reorganization of the dermis with an increase in collagen in the injured skin, due to the increase in the ratio of fibroblasts in the region. In this way, the treatment proves to be an interesting activator of the repair of the injured dermis. Macroscopic analyses of the clinical parameters of granulation and epithelialization showed better results in the control group with neomycin sulfate 5mg/g + bacitracin zincum 250 µl/g. We also observed that these same clinical and retraction parameters presented in the treatment groups with faveleira propolis are very similar to the groups treated with the control drugs Dexpanthenol 50 mg/g and Collagenase 0.6 IU/g, which suggests that faveleira propolis may have an important route of action in two moments of tissue repair after second-intention injury.

FINAL CONSIDERATIONS

Our data indicate that faveleira propolis has a similar action to the drugs neomycin sulfate 5mg/zinc bacitracin 250 µl/g, dexpanthenol 50 mg/g and collagenase 0.6 µl/g in second-intention skin lesions, especially in the control of inflammation and wound retraction in the initial phase. This is important for this type of lesion, which requires significant size reduction and tissue reorganization.

Keywords: Lesion repair, Skin lesions, Propolis Faveleira.

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