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Assessment of the efficiency of propolis on the Secondary Wound Healing of rabbits

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ABSTRACT— To investigate the Efficacy of propolis in the healing of cutaneous wounds of rabbits is studied on the basis of histopathological changes; Morphometric evaluations. The study was done at the Department of Oral and Maxillofacial Surgery, Dentistry College, Mosul University, this research included 25 domestic male rabbits1- 1.5 kg body weight and 1-2 years of age. The animals were divided into 2 groups, each animal, full-thickness skin wounds 3cm*3cm in diameter were created. One wound on each animal served as a control, the second wound on each animal propolis was applied daily, The wounds were investigated histopathological changes for wound healing after 3, 7, 14, 15, and 28 days. The data were obtained from the digital imaging analyses of wounds in the rabbit. There was no significant ($p<0.05$) between propolis-treated wounds and control wounds on day 7; wound area in propolis-treated, and control wounds on day 14; Within the healthy group, there were no significant differences in the propolis wound area on day 7, epithelization area on day 14; day 21and day 28. Within the propolis group, there were no significant differences in any of the evaluated parameters at any time point.so the wound site for group A1 with re-epithelialization (Score 3), creation of scar tissue constituted of mature fibrous tissue, angiogenesis with numerous blood vessels, and no inflammatory cell infiltration (score 0). Complete re-epithelialization (Score 4), creation of scar tissue, composed of mature fibrous tissue, angiogenesis with numerous blood vessels, and no infiltration of inflammatory cells were observed using H&E staining at 100X and 400X. (score 0). 100X, 400X H&E stain for group A2.also on days 14 (P-value=0.0293 *), 21 (P-value=0.767), and 28 (P-value= 1.00), significant scores of granulation tissue of rabbit skin wound healing process were found in propolis group A2 (P-value=0.0069 **) compared to the control group A1 (P-value=0.0074 **).

KEYWORDS: propolis; histopathological changes; Secondary Wound Healing of rabbits

1. INTRODUCTION

Propolis is available in a number of different hues, including green, red, and brown [1]. In order to be effective, a topical wound treatment solution must be biocompatible, nontoxic, and capable of promoting healing while not interfering with the body's natural healing process [2]. There is no particular therapy that can be used to enhance the healing of wounds that are difficult to heal [12].

In recent decades, scientists have been more interested in propolis because of its biological and pharmacological properties [3]. Because of this, propolis was selected for the present experiment in order to examine clinically its impact on secondary wound healing because it is a natural chemical with beneficial biological characteristics and no known negative effects on the body.

Propolis, according to [5], increased wound healing in a number of animal models, including wounds and burns, in addition to humans. According to the plant species used in their production, the health of the bees, the season, and the environment to which a honeybee hive is exposed, honey and propolis have a variety of

colors and tastes that vary from one another [4]. Different biological activity are caused by variances in their chemical composition [1]. Honey and propolis have been used in traditional medicine for thousands of years to cure burns and infected wounds, among other things [10]. Propolis was largely used as a mouth antiseptic and in the treatment of skin lesions, according to historical records. In ancient Egypt, propolis was also utilized in the process of mummification [13].

2. Materials and Methods

The study was done at the Department of Oral and Maxillofacial Surgery, Dentistry College, Mosul University, this research included 25 domestic male rabbits1- 1.5 kg body weight and 1-2 years of age. The animals were divided into 2 groups according to times of taking biopsies; On each animal, three full-thickness skin wounds 3cm*3cm in diameter were created(fig.1). One wound on each animal served as a control, the second wound on each animal propolis was applied daily, The wounds were investigated immunohistochemically for wound healing after 3, 7, 14, 15, and 28 days.

Figure (1): skin wounds 3cm*3cm in diameter

3. Results

On days 14 (P-value=0.0293 *), 21 (P-value=0.767), and 28 (P-value= 1.00), significant scores of granulation tissue of rabbit skin wound healing process were found in propolis group A2(PROPOLIS GROUP) (P-value=0.0069 **) and Mebo ointment group A2 (P-value=0.0069 **) compared to the control group A1 (P-value=0.0074 **) (Table -1).

Table (1): The scores of granulation tissue of rabbit skin wound healing process

Periods (Days)	A1 group (control)	A2(PROPOLIS GROUP) group (Propolis cream)	P-value
3 days	0	0	1.00

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7 days	**1**	**1**	**0.502**
14 days	**3**	**2**	**0.721**
21 days	**1**	**0**	**0.767**
28 days	**0**	**0**	**1.00**
P-value	**0.0074 ****	**0.0293 ***	**---**
*** (P≤0.05), ** (P≤0.01) **, NS: Non-Significant.**			

Table (2) showed on days 14 (0.0293 *), 21 (P-value=0.767), and 28 (P-value= 1.00), there were significant scores of granulation tissue of rabbit skin wound healing process in propolis group A2(PROPOLIS GROUP) (P-value=0.0069 **) and Mebo ointment group A2 (P-value=0.0069 **) compared to the control group A1 (P-value=0.0074 **).

Table 2: The scores of the inflammatory response of rabbit skin wound healing process

Periods (Days)	A1 group (control)	A2(PROPOLIS GROUP) group (Propolis cream)	P-value
3 days	**3**	**3**	**1.00 NS**
7 days	**1**	**2**	**0.781 NS**
14 days	**1** **1**	**2** **1**	**0.623 NS**
21 days	**1**	**0**	**0.609 NS**
28 days	**0**	**0**	**1.00 NS**
P-value	**0.0086 ****	**0.0083 ****	**---**
**** (P≤0.01), NS: Non-Significant.**			

Results in table (3) showed on days 14 (P-value=0.702), 21 (P-value=0.661), and 28 (P-value=0.817), wound reepithelialization was greater in the the propolis group A2(PROPOLIS GROUP) and Mebo ointment group A2 than in the control group A1 (P-value=0.0057 **).

Table (3): The scores of re-epithelialization of rabbit skin wound healing process

Periods (Days)	A1 group (control)	A2(PROPOLIS GROUP) group (Propolis cream)	P-value

3 days	**0**	**0**	**1.00**
7 days	**1**	**1**	**0.735**
14 days	**3**	**2**	**0.702**
21 days	**3**	**2**	**0.661**
28 days	**3**	**3**	**0.817**
P-value	**0.0057 ****	**0.0059 ****	**---**
**** (P≤0.01), NS: Non-Significant.**			

The data was obtained from the digital imaging analyses of wounds in the rabbit. There was no significant ($p<0.05$) between propolis-treated wounds and control wounds on day 7; wound area in propolis-treated, and control wounds on day 14; Within the healthy group, there were no significant differences in the propolis wound area on day 7, epithelization area on day 14; day 21and day 28. Within the propolis group, there were no significant differences in any of the evaluated parameters at any time point tables (4).

Table (4): comparative scores of granulation tissue, inflammatory response and re-epithelialization of rabbit skin wound healing process for the group (Propolis cream) with control

Periods (Days)	**Propolis cream group**			**Control group**		
	granulation tissue	**inflammatory response**	**re-epithelialization**	**granulation tissue**	**inflammatory response**	**re-epithelialization**
3 days	**0**	**3**	**0**	**0**	**3**	**0**
7 days	**1**	**2**	**1**	**1**	**1**	**1**
14 days	**2**	**2**	**2**	**3**	**1**	**3**
21 days	**0**	**0**	**2**	**1**	**1**	**3**
28 days	**0**	**0**	**3**	**0**	**0**	**3**
P-value						

In Figure (3) depicted the wound site for group A1 with re-epithelialization (Score 3), creation of scar tissue constituted of mature fibrous tissue, angiogenesis with numerous blood vessels, and no inflammatory cell infiltration (score 0). Complete re-epithelialization (Score 4), creation of scar tissue, composed of mature fibrous tissue, angiogenesis with numerous blood vessels, and no infiltration of inflammatory cells were

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observed using H&E staining at 100X and 400X. (score 0). 100X, 400X H&E stain for group A2(PROPOLIS GROUP) (fg.3, 4, 5 and 6).

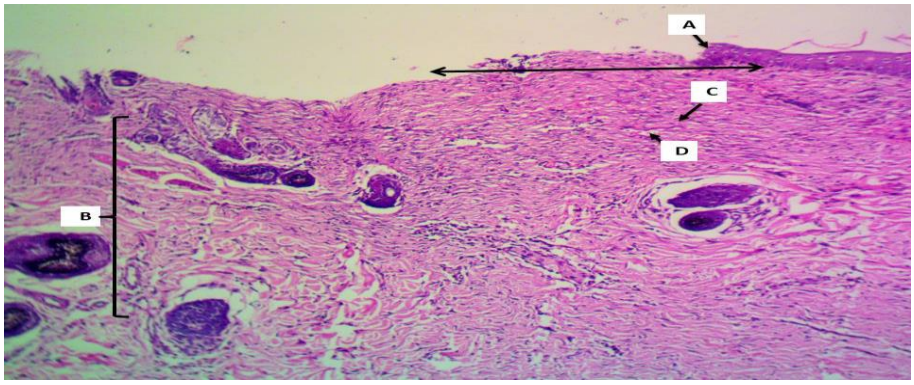

Figure (3): photomicrograph of rabbit skin of A1 (28 days) …….. group shows the site of wound (↔) with re-epithelialization (Score 3) (A), formation of scar tissue (B) composed of mature fibrous tissue (C), angiogenesis with highly blood vessels (D) without infiltration of inflammatory cells (score 0). H&E stain, 100X.

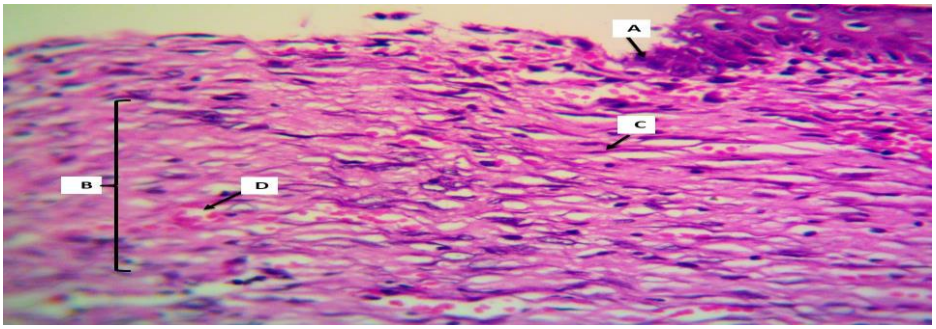

Figure (4-): photomicrograph of rabbit skin of A1 (28 days) …….. group shows the site of wound (↔) with re-epithelialization (Score 3) (A), formation of scar tissue (B) composed of mature fibrous tissue (C), angiogenesis with highly blood vessels (D) without infiltration of inflammatory cells (score 0). H&E stain, 400X

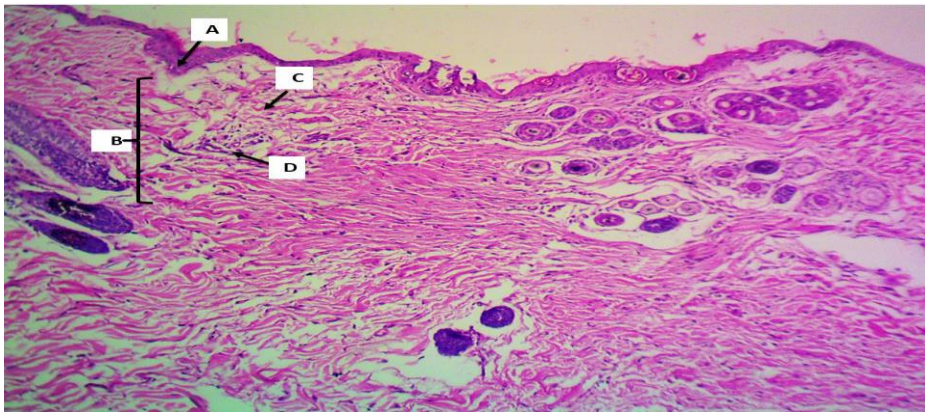

Figure (5): photomicrograph of rabbit skin of A2(PROPOLIS GROUP) (28 days) …….. group shows complete re-epithelialization (Score 4) (A), formation of scar tissue (B) composed of mature fibrous tissue (C), angiogenesis with highly blood vessels (D) without infiltration of inflammatory cells (score 0). H&E stain, 100X

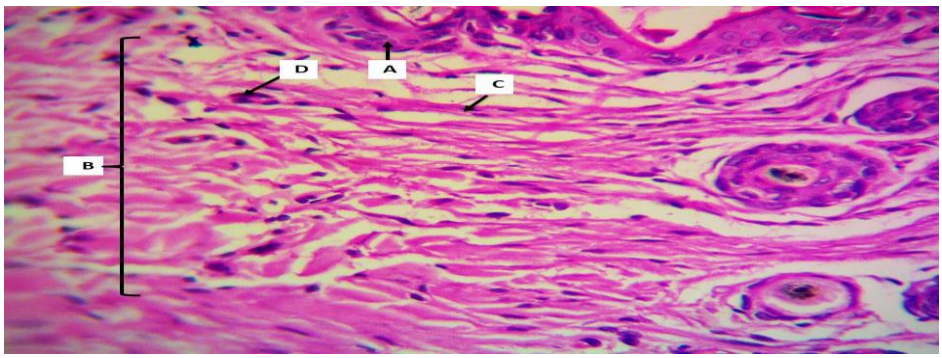

Figure (6): photomicrograph of rabbit skin of A2(PROPOLIS GROUP) (28 days) …….. group shows complete re-epithelialization (Score 4) (A), formation of scar tissue (B) composed of mature fibrous tissue (C), angiogenesis with highly blood vessels (D) without infiltration of inflammatory cells (score 0). H&E stain, 400X.

On days 14 (P-value=0.0293 *), 21 (P-value=0.767), and 28 (P-value= 1.00), significant scores of granulation tissue of rabbit skin wound healing process were found in propolis group A2(PROPOLIS GROUP) (P-value=0.0069 **) compared to the control group A1 (P-value=0.0074 **) (Table 1).

4. Discussion

The bunnies lived to see the end of the experiment. There were no mortality or clinical symptoms in any of the animals. The rabbits' bodies did not change considerably as a result of the various products evaluated.

According to the notion, propolis may aid in wound healing. a Propolis was chosen to symbolize a variety of wound therapies. propolis were used to represent synthetic sources, The healing process for wound treatments was evaluated using clinical wound parameters (inflammatory symptoms, blood vessel congestion, exudation, infection, and granulation tissue), wound contraction, and histologic features.

According to the notion, propolis may aid in wound healing. a Propolis was chosen to symbolize a variety of wound therapies.

Many previous studies showed Propolis-treated wounds healed effectively, with no signs of inflammation, exudation, infection, or granulation tissue. Granulation and epithelial tissue growth, on the other hand, took a long time to accomplish. The role of propolis in the healing process is still contested, however, it's generally linked to antibacterial properties, free radicals, and metabolic stimulation rather than direct tissue regeneration [9].

Also, early granulation tissue production with good epithelization, cellular neovascularization, and fibroblasts. This finding is predicted to contribute to propolis' healing properties as a result of a range of mechanisms, including propolis' antibacterial properties, which are largely related to the flavonoid content, particularly the presence of pinocembrin, galanga, and pinobanksin. Pinocembrin has been found to have antifungal properties. Two additional compounds with well-known characteristics are ester coumaric and caffeic acids [6], [14].

The anti-inflammatory and antioxidant properties of propolis are most likely responsible for its wound-healing abilities. Propolis or its active constituent, caffeic acid phenethyl ester (CAPE), reduces the protein concentration of pro-inflammatory proteinase, cyclooxygenase (COX) activity, and prostaglandins, resulting in immune cell and phagocyte stimulation [11], [8]. Propolis has long been used to heal burns and

wounds because of its antibacterial, anti-inflammatory, and antioxidant qualities [7]. As a result, we investigated whether propolis could aid wound healing by reducing inflammation in the nasal mucosa and hastening epithelial closure. Propolis, a resin-like substance changed by bee enzymes, is collected by honeybees. [13], Because Propolis is used to protect beehives and contains antibacterial, anti-inflammatory, and immunomodulatory characteristics. Because skin wound healing is a complex and well-organized process, it was hypothesized that propolis could be effective in wound treatment.

5. Conclusion

Propolis improves wound healing. Wound re-epithelialization and closure were accelerated much faster.

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