

Evaluation of the Effectiveness of Propolis Compared with Honey on Second Intention Wound Healing in the Equine

¹Abu-Ahmed, Howaida, ¹R.E. Abdel-Wahed, ¹M.H. El-Kammar and ²M.S. El-Neweshy

¹Department of Surgery, Faculty of Veterinary Medicine,
Alexandria University, Behira province, Rashid, Edfina, Egypt

²Department of Pathology, Faculty of Veterinary Medicine,
Alexandria University, Behira province, Rashid, Edfina, Egypt

Abstract: In the recent years, the search for natural compounds stimulate tissue repair has gained high importance. Therefore, the current study evaluated the effectiveness of propolis extract, natural wound dressing, for treatment of equine old wounds. Thirty five old wounds in neck and shoulder regions met with in 18 horses and 14 donkeys were dressed with propolis. Clinical wound characters following propolis treatment proved good. They showed marked reduction in wound dimensions and formation of healthy scars. On the other hand, fifteen surgically induced full-thicknesses skin wounds at the shoulder region in 5 donkeys (3 wounds / each animal) were dressed for 3 weeks with propolis, honey and saline, respectively (5 wounds / each treatment). Clinical wound characters following treatment with propolis proved better. According to histological parameters, the propolis treated wounds proved advantageous than honey and saline treated wounds and showed moderate to complete thick vascular granulation tissue with more fibroblasts and collagen deposition, mild cellular invasion and complete epithelial migration with no infection. In conclusion, propolis as compared with honey is beneficial treatment for equine old wounds.

Key words: Propolis • Honey • Equine • Old wounds • Dressing • Healing

INTRODUCTION

Skin wound healing is a complex and well organized process involving a sequence of continuous and overlapping events including inflammation, re-epithelization and dermal reconstitution, wound contraction and remodeling [1, 2]. The search for natural compounds to stimulate tissue repair has gained importance in the recent years aiming development of non-toxic formulations for wound treatment due to their easy application, low cost and bactericidal/bacteriostatic effect [3]. For centuries, honey has been used as an effective remedy for wounds. It is a popular sweetener and a common household product throughout the world [4]. Propolis, or bee glue, is a brownish resinous material collected by worker bees from the leaf buds of numerous tree species like birch, poplar, pine, alder, willow and palm [5]. The term propolis (Russian Penicillin) is derived from the Greek word "pro" = in front of or at the entrance to and "polis" = community or city and the whole word

means a substance in defense of the hive. It was used at the time of Egyptian and Greek civilizations which recognized its healing qualities. Hippocrates, the founder of modern medicine, used propolis for healing sores and ulcers internally and externally. This non-toxic resinous substance was classified into 12 types according to physico-chemical properties and related to geographic locations [5, 6]. The components of propolis have antibacterial, antifungal and antiviral activity.

In addition, propolis has anti-inflammatory and immunomodulatory activities [7]. The present study is designed to investigate the benefits of propolis for treatment of old wounds in equine.

MATERIALS AND METHODS

Source of Propolis: Propolis was collected from bee hives as natural and raw material, dried, grinded and purified according to the method described by Zhe *et al.* [8]. Propolis was used in form of powder (Figure 1).



Fig. 1: Propolis powder after purification



Fig. 2 a, b and c: Clinically-treated wounds with propolis powder: a and b) Old wounds during wound debridement before treatment for removal of unhealthy and necrotic tissues. c) Freshening of the wound

Treatment of Old Wounds: 32 animals (18 horses and 14 donkeys) aging 4-10 years were admitted to the clinic of Faculty of Veterinary Medicine, Alexandria University, Egypt, early in 2012. Upon examination, they were suffering from 35 old wounds with variable sizes and shapes in neck (13) and shoulder regions (22) with unhealthy tissues and infection of wound surface. Wound dimensions (length and width) were measured. The wound area was prepared routinely. Surgical debridement before treatment was performed for removal of unhealthy tissues (Figures, 2 a and b) and freshening of the wound (Figure, 2 c). Hemorrhage was controlled by packing. The wound surface was then covered with a thin layer of propolis powder. The wound was then protected by sterile piece of gauze followed by a protective bandage. The bandage was changed day after day in the first ten days, every fourth day for two weeks and then once weekly till the complete healing took place. The frequency of changing the dressing depended on how rapidly the propolis was diluted with the exudates. Wound dimensions were again calculated at end of each week.

Treatment of Surgical Wounds: Five apparently healthy donkeys (4-7 years old) were used to induce 15 full-thickness skin wounds in shoulder region (3 wounds in each donkey). These wounds were conducted and treated to investigate the effect of propolis on healing. The effects were evaluated histologically comparing with

the effects of honey as well as that of 0.9% saline (control). Animals were physically and chemically controlled in addition to local infiltration analgesia using 10 ml of 2% xylocaine HCl at the site of skin incision. Following aseptic preparation of the wound area a skin flap including the subcutis measuring 4 cm length and 4 cm width was excised. Hemorrhage was controlled. The three wounds of each animal were treated with propolis powder, honey and saline, respectively. Propolis was poured on wound surface in form of a thin layer, while honey or saline were used as a soaked piece of gauze covering the wound surface. Thereafter, wounds were handled in the same manner as with clinical animals admitted with old wounds. Animals were kept under observation till wound healing took place.

Evaluation of wound healing based on; wound contraction % and histologic features. Mean reduction in wound dimensions (length and width) was determined at day 7, day 14 and day 21 for calculating wound contraction % according to the equation described by Ramsey *et al.* [9].

$$\text{Wound Contraction (WC \%)} = (W_0 - W_1) / W_0 * 100$$

where:

W₀ = The initial wound measurement (1st measurement in cm),

W₁ = The wound measurement on day of measurement (2nd measurement in cm).

Table 1: Scores and correlated pathological criteria

Score	Criteria
1-3	- None to minimal cell accumulation. - No granulation tissues or epithelial travel.
4-6	- Thin, immature granulation that is dominated by inflammatory cells but has few fibroblasts, capillaries or collagen deposition. - Minimal epithelial migration.
7-9	- Moderately thick granulation tissues can range from being dominated by inflammatory cells to more fibroblasts and collagen deposition. - Extensive new vascularization. - Epithelium can range from minimal to moderate migration.
10-12	- Thick, vascular granulation tissues dominated by fibroblasts and extensive collagen deposition. - Epithelium partially to completely covering the wound.

Skin specimens were collected from the surgically induced wounds, after 3 weeks and fixed in 10% neutral buffered formalin, prepared and stained by H and E for histopathological examination. A histological scores ranging from 1 to 12 were recorded (Table 1). The scoring based on the degree of cellular invasion, granulation tissue formation, vascularity and re-epithelialization [10].

RESULTS

Old Wound Healing in Clinical Cases Treated with Propolis: Healing of old wounds of clinical cases treated with propolis took place in a period of time ranged from 3-5 weeks. Neither adhesion (to damage the granulating surface) nor bleeding was established following dressing changing (Figure 3).

After one week of treatment, 27 of the 35 clinically treated wounds with propolis showed clean healthy bright red surface with no infection and marked decrease in wound dimensions (Figure 4). After 2-3 weeks of treatment, wound sizes showed marked reduction and significant increase in wound contraction % with granulation tissue formation (Figure 5a and b and Table 2).

Surgical Wound Healing in Donkeys Treated with Propolis, Honey and Saline: Healing of surgically induced wounds in donkeys took place within one month, with some difference, in the three treated groups (propolis, honey and saline).

Clinical wound characters following treatment with propolis powder showed no exudates and the granulation tissues filled the wound cavity in the first week (Figure 6a). By end of the second week, wound markedly contracted and invaded by epithelium from the periphery (Figure 6 b). The honey-treated wounds showed some exudates and the granulation tissues were found in the periphery (Figure 7 a and b). On the other hand, the saline-treated wounds showed some inflammatory signs

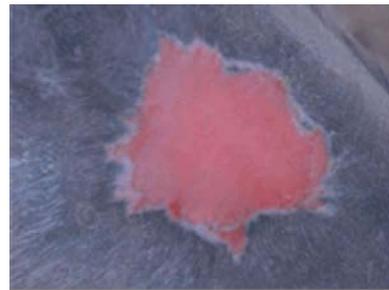


Fig. 3: Clinically treated wound showed smooth surface, one week after propolis treatment



Fig. 4: Clinically-treated wound showed clean and healthy bright red surface with no infection, 18 days after propolis treatment

and bleeding tendency was detected when changing bandages. Epithelialization appeared in the periphery (Figure 8 a and b).

Throughout the period of treatment, there was variation in mean reduction of wound dimensions (Figure, 4, 5, 6 b and 7 b). The percent of wound contraction (WC %) showed varieties responding to each treatment (Tables, 2 and 3).

Histopathological Findings of Surgical Wounds Treated with Propolis, Honey and Saline: As compared with the histologic parameters following normal saline which included mild re-epithelialization, mild granulation tissue

Table 2: Mean ± SD of wound dimensions for clinical old wounds in horses and donkeys treated with propolis

Animals		At arrival (W x L)	After week (W x L)	After 2 weeks (W x L)	After 3 weeks (W x L)
Horses	- Dimensions	5±0.4 x 6.1 ±0.7	3.5±0.3 x 4±0.2	2±0.1 x 1.6 ±0.06	0.9±0.03 x 0.3±0.01
	- WC %	-	30	42.9	55
Donkeys	- Dimensions	4.3±0.6 x 4.1±0.2	3.2±0.3 x 3.2±0.3	1.9±0.05 x 2.2±0.02	0.3±0.2x 0.7±0.02
	- WC %	-	25.6	40.6	84.2

Wound dimensions are measured in centimeter

Table 3: Mean ± SD of wound dimensions for surgically induced wounds in donkeys treated with propolis, honey and saline

Substances		After week (WxL)	After 2 weeks (WxL)	After 3 weeks (WxL)
Propolis	- Dimensions	3.2±0.3 x 2.8±0.2	1.8±0.2 x 2.1±0.1	Complete healing
	- WC %	20	43.8	100
Honey	- Dimensions	3.3±0.3 x 3.1±0.4	2.1±0.2 x 2.3±0.03	0.9±0.06 x 1.1±0.03
	- WC %	17.5	36.4	57.1
Saline	- Dimensions	3.5±0.5 x 3.2±0.1	2.9±0.4 x 2.6±0.2	1.8±0.3 x 1.7±0.07
	- WC %	12.5	17.1	37.9

Basal wound dimensions = 4x4 cm

Table 4: Histological evaluation of wound healing parameters after 3 weeks following different treatments in donkeys

Parameter	Saline	Propolis	Honey
Re-epithelialization	+	+++	++
Cellular invasion	++	+	+
Granulation tissue formation	+	++	++
Vascularity	+	++	++
Infection	-	-	-
Wound healing score (1-12)	4	10	7

+ Mild ++ Moderate +++ Severe or complete

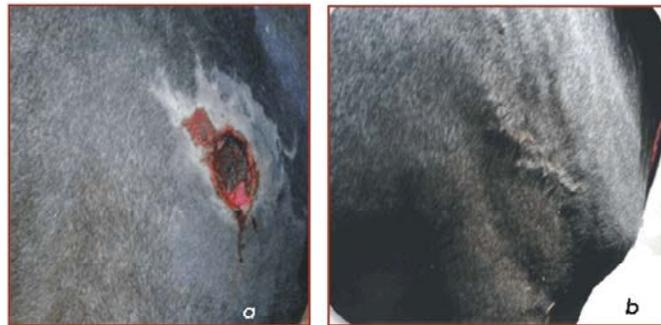


Fig. 5: Clinically-treated wounds with propolis powder: a) Old wound in shoulder, 14 days after propolis treatment showed significant decrease in wound size with granulation tissue formation. b) The same wound, one month later showed complete healing



Fig. 6: Surgically-induced wound in shoulder region of donkeys treated with propolis: a) No exudates and the granulation tissues filled the wound cavity in the first week. b) By end of the second week, marked wound contraction and with invasion of epithelium from the periphery

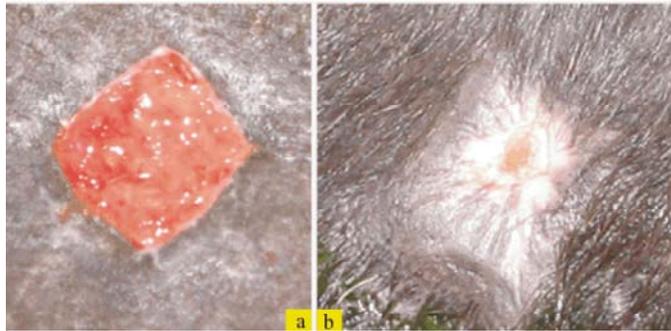


Fig. 7: Surgically-induced wound in shoulder region of donkeys treated with honey: a) Some exudates and the granulation tissues found in periphery. b) By end of the second week, the wound began to contract with moderate epithelialization from the periphery



Fig. 8: Surgically-induced wound in shoulder region of donkeys treated with saline: a) Some inflammatory signs and bleeding. b) By end of the second week, epithelialization appeared in the periphery

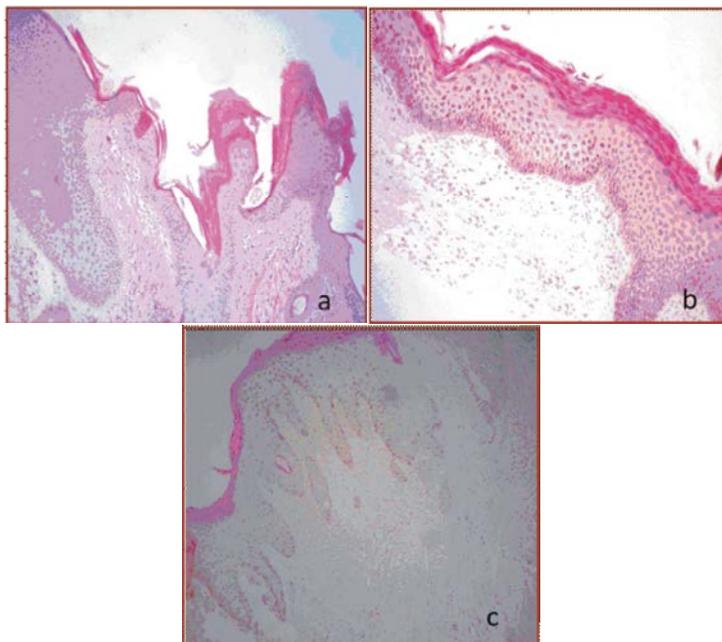


Fig. 11: a) Normal skin histology, ch. By epidermis (keratinized stratified squamous epithelium) with underlying dermis. H&E (X 100) .b) Skin wound, 3 weeks after treatment with proplis powder, ch. By moderately thick granulation tissue with more fibroblasts and collagen deposition with complete epithelial migration. H&E (X 250) .c) Skin wound, 3 weeks after treatment with noney ch. By thick, vascular granulation tissue dominated by fibroblasts and extensive collagen deposition. Epithelium completely covering the wound. H&E (X 250).

formation and vascularity with moderate cellular invasion; wound treatment with propolis powder or with honey showed complete re-epithelialization, mild cellular invasion, moderate to complete granulation tissue formation and vascularity with no infection (Table 4).

As compared with the normal skin histology (Figure, 11 a), skin wound healing after 3 weeks from treatment with propolis powder and with honey proved advantageous than that following saline. They showed moderately thick to thick vascular granulation tissues with more fibroblasts and collagen deposition and complete epithelial migration after propolis and honey, respectively (Figures., 11 b and c).

DISCUSSION

As propolis (bee glue) is used for defense against bee hives, in addition to its possession of anti-microbial, anti-inflammatory and immunomodulatory activities and as skin wound healing is a complex and well organized process, it was therefore hypothesized that propolis may have an importance in wound management. Evaluation of healing process for wound treatments was based on; clinical wound parameters (inflammatory signs, bleeding tendency, exudation, infection and granulation tissue), wound contraction and histologic features.

In this investigation, wounds treated with propolis provided good healing parameters, characterized by absence of inflammatory signs, exudation and infection. Meanwhile, granulation and epithelial tissue formation were moderate to complete. The effective action of the bee propolis during the healing process is still a controversial issue and in general is associated to its antimicrobial characteristics, free radicals and stimulating of metabolism rather than direct tissue regeneration [5, 11, 12]. The complexity of the propolis extract to its multiplicity of compounds did not allow us identifying the substance(s) responsible for the healing effectiveness. However, this characteristic may be attributed to presence of flavonoids due to their bactericide and antiviral behaviors [13]. The propolis healing property may also be due to substances derived from fatty acids, terpenoid compounds, steroids, vitamins and mineral salts which are present in propolis compounds [11]. On other hand, wounds treated with saline showed incidence of some exudes and infection. These may be due to presence of microbial agent. Propolis, by its biological contents, had a role in solution of such problem by improvement of wound healing preventing infection and stimulating

granulation and epithelialization. The beneficial results of honey indicated that honey itself has positive effects on wound healing. These effects may be due to the high level of glycin, methionin and prolin in honey. These constituents play a role in collagen formation [14]. This advantage may be attributed to the nutritive value of honey. Degree of inflammation and swelling usually reduced after treatment with honey [15, 16]. Propolis handling proved easy to be applied and to be removed during wound dressing. Neither adhesion to damage the granulating surface nor bleeding was established. These simulate the characteristic feature of honey dressing [16-19]. Although good wound healing could be achieved by many treatments, the dressing of choice is that being available, easily prepared and applied, economic and safe. Herbal dressing like chamomile and marjoram achieved good results [20]. Their availability and preparation were so difficult. Centripetal movement of the skin over the granulation surface is meant as wound contraction, resulting in stretching and thinning of the skin as it is pulled towards the center of the wound. Later in the healing process the skin will thicken by a process called intussusceptive growth [21]. In the present study, propolis was found to have positive effects on wound healing by accelerating wound contraction through marked reduction in wound dimensions and higher wound contraction %. Castaldo and Capsso [5] supported these findings. The histologic features following propolis dressing proved excellent characterized by thick vascular granulation tissues, more fibroblast and collagen deposition and epithelial migration. Meagher [22] and Less *et al.* [23] considered these items as parameters up on which the histologic feature is judged as good and the healing process is considered acceptable.

CONCLUSION

It could be concluded that using of propolis accelerates wound healing and it is therefore, advisable to be used as a dressing for equine wound management due to its clinical value and easy application.

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