Surgical and Histological Evaluation of the Effectiveness of Propolis on Wound Healing

Abdel-Wahed RE, El-Kammar MH, Korittum AS and Edrees, IR

Dept. Surgery, Fac. Vet. Medicine, Alexandria University, Egypt

Key words: Propolis, Dogs, Wounds, Dressing, Healing, Histological evaluation.

ABSTRACT: The search for natural compounds stimulate tissue repair is a target. The current experimental investigation evaluated the effectiveness of propolis compared with honey/cod liver oil mixture, Mebo and panthenol, for treatment of surgically induced full-thickness skin wounds in the back region in dogs. Clinical wound characters following propolis treatment proved good and showed marked reduction in wound dimension and formation of healthy scar. The quantification of observation concerning the histological parameters involved in wound healing indicated that the propolis treated wounds proved advantageous. It could be concluded that propolis accelerates wound healing and it is advisable to be used for wound dressing due to its clinical value and easy application.

Corresponding Author: Islam Edress, dr memo_210@yahoo.com

1. INTRODUCTION

The search for natural compounds to stimulate tissue repair has gained importance in the recent years aiming development of non-toxic formulation for wound treatment due to their easy application, low cost, and bactericidal/bacteriostatic effect (Mathews and Binnington, 2002). 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 (Coulombe, 1997 and Stadelman et al., 1998). 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 (Bansal et al., 2005). 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 (Castaldo and Capasso, 2002). This non-toxic resinous substance was classified into 12 types according to physico-chemical properties and related to geographic locations (Wander, 1995 and Castaldo and Capasso, 2002). The components of propolis have a multiple effects on bacteria, fungi and viruses. In addition, propolis and its components have anti-inflammatory and immunomodulatory activities (Bankova and De Castro, 2000). A recent study conducted by Abo-Bakr (2011) provided the clinical importance of propolis in wound healing in equine wounds. The present experimental study is designed to evaluate the effects of propolis in wound healing and to compare its effects with that of honey/cod liver oil mixture, mebo and panthenol in dog wounds.
2- Materials and Methods:
2.1- Animals and Wounds:
Twenty apparently healthy mongrel dogs (2-3 years old and weighing 15-18 kg) were used in the present experimental investigation. They were physically controlled in sternal recumbent position after sedation by xylazine HCl injected intramuscular in a dose rate of 1 mg/ kg body weight. Local infiltration analgesia using 2% xylocaine HCl was injected at the site of skin incision. After routine aseptic preparation of the wound area in each dog, 5 neighboring rectangular full-thickness skin wounds measuring 2 cm width and 3 cm length for each were conducted (Fig., 1). Hemorrhage was controlled by pressure on the wound surface for 2 minutes. The wounds were bandaged with adhesive tape to protect them from contamination and flies for 24 hours (Fig., 2). Immediately before treatment application, each wound was washed with 10% povidone iodine and irrigated with distilled water as a start point.

2.2- Wound management:
Wounds were treated in the following manner; each dog (5 wounds) received all treatments. One wound treated with 0.9% saline solution as a control, one wound treated with propolis powder, one wound with MEBO® (Moist Exposed Burn Ointment), one wound with panthenol and the last wound was treated with honey/cod liver oil mixture. Application of the medicament was carried out by direct topical application over the wound surface (Fig., 3) that was covered with sterile piece of gauze followed by a protective bandage changed day after day in the first week, every fourth day for two weeks and then once weekly till complete healing took place.

2.3- Wound Evaluation:
Evaluation of wound healing based on; clinical wound characters, 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. (2005).

\[ \text{Wound Contraction (WC %)} = \frac{W_0 - W_1}{W_0} \times 100 \]

Where:
- \( W_0 \) = the initial wound measurement (1st measurement in cm).
- \( W_1 \) = the wound measurement on day of measurement (2nd measurement in cm).

2.3- Histological evaluation:
Skin wound biopsies were obtained after 24 hours, 3 days, 6 days and 16 days for histological evaluation. They were prepared, fixed in 10% buffered formalin, processed and stained with hematoxylin and eosin (H & E) for light microscopic examination according to Bancroft et al. (2013). The degree of cellular infiltration, collagen production, neovascularization and the thickness of epithelium over the wound were scored according to a scoring system described by Karayannopoulou et al. (2011).

For the degree of cellular infiltration, sections were assigned a score for the number of inflammatory cells (neutrophils, lymphocytes, plasma cells, macrophages, eosinophils, and mast cells) detected per high power field (HPF) (X400). A score of 0 means ≤ 3 inflammatory cells, score 1 means 4 to 10 inflammatory cells, 2 means 11 to 20 inflammatory cells, 3 means 21 to 30 inflammatory cells, 4 means 31 to 40 inflammatory cells, and score 5 means ≥ 41 inflammatory cells. For collagen production, sections were assigned scores as follows: score 0 = normal; 1 = mild increase; 2 = mild to moderate increase; 3 = moderate increase; 4 = moderate to marked increase and score 5 = marked increase that means extensive or involves deep tissues. The number of blood vessels and capillary buds (as indicator of neovascularization or angiogenesis) were evaluated in 5 HPFs per section. Moreover, sections were
assigned an angiogenesis score of 1 means presence of 3 to 10 new vessels, 2 = 11 to 30 new vessels, while score 3 means presence of ≥ 31 new vessels per HPF. The thickness of epithelium over the wound was also compared with that of the normal epidermis of excised skin on day 0 (control specimen) and assigned a score 0 (thickness similar to that of normal epithelium), 1 (slightly increased thickness), 2 (moderately increased thickness), and 3 (markedly increased thickness).

2.4- Statistical analysis:

The collected data in the present investigation are subjected to statistical analysis using One-Way ANOVA with Duncan's multiple range tests (SAS, 2006).

3- Results:

3.1- Clinical wound characters:
Throughout the period of treatment, clinical wound parameters including inflammatory signs, bleeding tendency, exudation, infection and granulation tissue showed low significant difference between different drugs. Wounds treated with propolis provided good healing parameters, characterized by absence of inflammatory signs, exudation and infection (Fig.4a, b, c). Granulation and epithelial tissue formation were moderate to complete and rapidly seen after propolis (Figs., 5 & 6).

3.2- Wound contraction:
Change in the wound dimensions varied according to different treatments. There was marked variation in mean
reduction of wound width (MRW) and mean reduction in wound length (MRL). The percent of wound contraction showed great varieties responding to each treatment. Mean reduction in the wound length and wound width as well as mean wound contraction % are summarized in Table (1).

3.3- Histologic features:

During the proliferative stage, after 3 days, the control group showed focal re-epithelization with absence of underlying neovascularization (Fig., 7b). Panthenol dressing group showed covering epithelial cells proliferate and 'crawl' atop the wound bed, providing cover for the new tissue with underlying neovascularization and considerable number of inflammatory cells mainly neutrophil (Fig., 7c). Mebo dressing group characterized by covering epithelial cells proliferate and 'crawl' atop the wound bed, providing cover for the new tissue with underlying neovascularization and few inflammatory cells (Fig., 7d). Propolis dressing group showed covering epithelial cells proliferate and 'crawl' up to the wound bed, providing cover for the new tissue with marked underlying neovascularization and few inflammatory cells mainly macrophages (Fig., 7e). Honey and Cod liver oil dressing group showed covering epithelial cells proliferate and 'crawl' atop the wound bed, providing cover for the new tissue with marked underlying neovascularization and considerable numbers of inflammatory cells mainly macrophage (Fig., 7f).

During the wound contraction, after 16 days, the control group showed complete epithelization with rete ridges formation under scab formation with underlying granulation tissue formation featuring fibroblast and few collagen perpendicular to new blood vessel and parallel to wound surface (Fig., 8b). Panthenol and Mebo dressing group
showed complete epithelization with rete ridges formation with underlying granulation tissue formation featuring fibroblast and collagen perpendicular to new blood vessel and parallel to wound surface (Fig., 8c & d). Propolis dressing group showed complete epithelization with rete ridges formation with underlying fibrous tissue where fibroblast and collagen bundles (devoid new blood vessels) parallel to wound surface (inset; X400) (Fig., 8e). Honey and Cod liver oil dressing group showed complete epithelization with rete ridges formation with underlying granulation tissue formation featuring fibroblast and collagen perpendicular to new blood vessel and parallel to wound surface (Fig., 8f). The quantification of observations concerning the histological parameters (variables) involved in wound healing allowed detection of a number of differences between the 5 groups, overall favoring the group treated with wound dressing (Table, 2 and histograms 1-4).

**Table (1): Wound dimensions and wound contraction (WC) percent for surgically induced wounds in dogs treated with propolis, Mebo, panthenol, honey/cod liver oil mixture and saline**

<table>
<thead>
<tr>
<th>Substances</th>
<th>After week (W x L)</th>
<th>After 2 weeks (W x L)</th>
<th>After 3 weeks (W x L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propolis</td>
<td>- Dimensions (cm)</td>
<td>2.2 ± 0.3 x 1.4 ± 0.2</td>
<td>1.6 ± 0.2 x 0.7 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>- WC (%)</td>
<td>20</td>
<td>43.8</td>
</tr>
<tr>
<td>MEBO</td>
<td>- Dimensions (cm)</td>
<td>2.4 ± 0.3 x 1.7 ± 0.4</td>
<td>1.8 ± 0.2 x 1.1 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>- WC (%)</td>
<td>17.5</td>
<td>36.4</td>
</tr>
<tr>
<td>Panthenol</td>
<td>- Dimensions (cm)</td>
<td>2.5 ± 0.5 x 1.8 ± 0.1</td>
<td>1.9 ± 0.4 x 1.3 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>- WC (%)</td>
<td>12.5</td>
<td>17.1</td>
</tr>
<tr>
<td>Honey/Cod liver oil</td>
<td>- Dimensions (cm)</td>
<td>2.3 ± 0.4 x 1.6 ± 0.2</td>
<td>1.9 ± 0.4 x 0.9 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>- WC (%)</td>
<td>19</td>
<td>41.4</td>
</tr>
<tr>
<td>Saline</td>
<td>- Dimensions (cm)</td>
<td>2.8 ± 0.4 x 1.9 ± 0.3</td>
<td>2.1 ± 0.4 x 1.6 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>- WC (%)</td>
<td>11.4</td>
<td>15.4</td>
</tr>
</tbody>
</table>

- Basal wound dimensions = 3 x 2 cm
- MEBO (Moist Exposed Burn Ointment).
- All values are expressed as Mean ± SE. The values indicated by different letters, within the same row, are significantly different (P < 0.05).
**Table (2): Changes in histological parameters during wound healing process**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Panthenol</th>
<th>Mebo</th>
<th>Propolis</th>
<th>Honey and cod liver oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellular infiltration</td>
<td>2.4±0.24 c</td>
<td>3.8±0.37 b</td>
<td>3.2±0.2 c</td>
<td>4.8±0.18 a</td>
<td>4.6±0.2 a</td>
</tr>
<tr>
<td>Neovascularization</td>
<td>1.2±0.2 d</td>
<td>2.0±0.31 d</td>
<td>2.2±0.18 c</td>
<td>2.8±0.2 a</td>
<td>2.6±0.24 b</td>
</tr>
<tr>
<td>Epithelium thickness over the wound</td>
<td>2.8±0.2 d</td>
<td>1.5±0.19 b</td>
<td>2.0±0.31 d</td>
<td>1.2±0.2 a</td>
<td>1.4±0.24 b</td>
</tr>
<tr>
<td>Collagen production</td>
<td>1.4±0.23 d</td>
<td>2.8±0.37 c</td>
<td>3.0±0.32 c</td>
<td>4.4±0.36 a</td>
<td>4.0±0.41 b</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SE. The values indicated by different letters, within the same row, are significantly different (P < 0.05).

4- Discussion:

Because of propolis is used for defense against bee hives, and due 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 hypothesized that propolis may have an importance in wound management. Selection of MEBO, panthenol, honey and cod liver oil in addition to propolis was to represent varieties of wound dressings. MEBO and panthenol represent the synthetic sources while honey and cod liver oil represent natural sources. 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 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 (Burdock, 1998, Marcucci, 2001 and Castaldo and Capasso, 2002). 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 feature may be attributed to presence of flavonoids due to their bactericide and antiviral behaviors (Havsteen, 2002). The propolis healing property may also be due to substances derived from fatty acids, terpeniol compounds, steroids, vitamins and mineral salts which are present in propolis compounds (Burdock, 1998). 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 glycine, methionin and prolin in honey.
Fig (7): Photomicrograph of skin wound healing in dog (after 3 days) stained with hematoxylin and eosin (H&E): (A) Normal skin histology (X40). (B): Control group without dressing showing focal re-epithelization (arrow; X40) with absence of underlying neovascularization (inset; X400). (C): Panthenol dressing group showing covering epithelial cells proliferate and 'crawl' atop the wound bed, providing cover for the new tissue (long arrow; X40) with underlying neovascularization (curved arrow) and considerable number of inflammatory cells mainly neutrophil (inset; X400). (D): Mebo dressing showing covering epithelial cells proliferate and 'crawl' atop the wound bed, providing cover for the new tissue (arrow; X40) with underlying neovascularization (curved arrow) and few inflammatory cells (inset; X400). (E): Propolis dressing group showing covering epithelial cells proliferate and 'crawl' atop the wound bed, providing cover for the new tissue (arrows; X40) with marked underlying neovascularization (curved arrow) and few inflammatory cells mainly macrophages (inset; X400). (F): Honey + Codliver oil dressing group showing covering epithelial cells proliferate and 'crawl' atop the wound bed, providing cover for the new tissue (arrow; X40) with marked underlying neovascularization (curved arrow) and considerable numbers of inflammatory cells mainly macrophage (inset; X400).
Fig (8): Photomicrograph of skin wound healing in dog (after 16 days) stained with hematoxylin and eosin (H&E): (A) Normal skin histology (X40). (B): Control group without dressing showing complete epithelization with rete ridges formation (thick arrow) under scab formation; with underlying granulation tissue formation featuring fibroblast and few collagen (thin arrows) perpendicular to new blood vessel (line, inset; X400) and parallel to wound surface. (C): Panthenol dressing group showing complete epithelization with rete ridges formation; with underlying granulation tissue formation featuring fibroblast and collagen (thin arrows) perpendicular to new blood vessel (line, inset; X400) and parallel to wound surface. (D): Mebo dressing showing complete epithelization with rete ridges formation; with underlying granulation tissue formation featuring fibroblast and collagen (thin arrows) perpendicular to new blood vessel (line, inset; X400) and parallel to wound surface. (E): Propolis dressing group showing complete epithelization with rete ridges formation; with underlying fibrous tissue where fibroblast and collagen bundles (devoid new blood vessels) parallel to wound surface (inset; X400). (F): Honey + Codliver oil dressing group showing complete epithelization with rete ridges formation; with underlying granulation tissue formation featuring fibroblast and collagen (thin arrows) perpendicular to new blood vessel (line, inset; X400) and parallel to wound surface.
These constituents play a role in collagen formation (Gupta et al., 1992). This advantage may be attributed to the nutritive value of honey. Degree of inflammation and swelling usually reduced after treatment with honey (Molan, 1998 and Subrahmanyan, 1998). 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 (Efem, 1998, Farouk et al., 1998 as well as Subrahmanyan, 1994 and 1998). Herbal dressing like chamomile and marjoram achieved good results (El-Faramawi, 2005). Their availability and preparation were so difficult. 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 (2002) 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 (1981) and Less et al. (1989) considered these items as
parameters up on which the histologic feature is judged as good and the healing process is considered acceptable. It could be concluded that propolis accelerates wound healing and is therefore, advisable to be used as a dressing for wound management due to its clinical value and easy application.

The authors express gratitude and appreciation to Dr. Mahmoud El-Newaishy, assistant professor of pathology, faculty of veterinary medicine, Alexandria University, for his help for completion of the part of histopathology.

5- REFERENCES
Faculty of Vet. Med., Alexandria University.