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The Potential Effect of Honey Varieties on Wound Healing Treatment in Dogs

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Abstract: A wound is a physical bodily injury resulting in the disruption of normal continuity of structures and wound healing is the restoration of the continuity. The objectives of the study to evaluate effects of different types of honey on wound healing in Dogs. Twenty-four healthy female dogs weighing between 8 to 12 kg were used in this study. The animals were randomly into four experimental groups with each group consisted of 6 animals over a 21-days period. After the creation of 4cm x 4cm open wound, Group I was control treated with Gentamycin ointment. Groups II, III and IV were treated with Different Types of Honey. On application, the honey was well accepted by the animals without any adverse reaction. On clinical examination, Groups II, III and IV showed bright beefy red color granulation tissue with angiogenesis when compared to Groups I. Mean percentage of epithelialistion, wound contraction and total healing were significantly better in Group II (92.43%). Honey can be a better wound healing biomaterial in dogs. It can be used as a less expensive skin substitute in order to stimulate and promote wound healing in animals.

Keywords: Honey, wound healing, Sidr honey, Arbutus unedo honey, Eucalyptus honey.

التأثير المحتمل لأصناف العسل على علاج التئام الجروح في الكلاب

المستخلص: الجرح هو إصابة جسدية جسدية تؤدي إلى تعطيل الاستمرارية الطبيعية للهياكل، ويعتبر شفاء الجرح بمثابة استعادة الاستمرارية. أهداف الدراسة لتقييم تأثير أنواع مختلفة من العسل على التئام الجروح في الكلاب. استخدمت في هذه الدراسة أربع وعشرون أنثى كلب سليمة يتراوح وزنها ما بين 8 إلى 12 كجم. تم تقسيم الحيوانات بشكل عشوائي إلى أربع مجموعات تجريبية بحيث تتكون كل مجموعة من 6 حيوانات على مدى 21 يومًا. بعد تكوين جرح مفتوح مقاس 4 سم × 4 سم، تم علاج المجموعة الأولى بمرهم الجنتاميسين. تم علاج المجموعات الثانية والثالثة والرابعة بأنواع مختلفة من العسل. عند الاستخدام، تم قبول العسل بشكل جيد من قبل الحيوانات دون أي رد فعل سلبي. في الفحص السريري، أظهرت المجموعات الثانية والثالثة والرابعة أنسجة حبيبية ذات لون أحمر ساطع مع تكوين الأوعية الدموية عند مقارنتها بالمجموعات الأولى. وكان متوسط النسبة المئوية للتشكل الظاهري وتقلص الجروح والشفاء الكلي أفضل بكثير في المجموعة الثانية (92.43٪). يمكن أن يكون العسل مادة حيوية أفضل لشفاء الجروح في الكلاب. ويمكن استخدامه كبديل أقل تكلفة للجلد من أجل تحفيز وتعزيز التئام الجروح في الحيوانات.

الكلمات المفتاحية: العسل، التئام الجروح، عسل السدر، عسل الكفور، عسل الحنون.

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INTRODUCTION

A wound is a physical bodily injury resulting in the disruption of normal continuity of structures and wound healing is the restoration of the continuity. The treatment of wounds has improved considerably in the past 30 years, and will continue to progress rapidly with the advancing technology and a greater understanding of chronic wounds (Ballard & Baxter, 2000; Khaled et al., 2018) The objectives of any wound management are relief of pain and distress to the animal, functional and cosmetic repair, economic and time efficient procedures and prompt decision making in the event of signs of delayed healing (Cockbill, 2002). In chronic wounds, the major focus of wound healing has been on the relationship between tissue destruction by excess inflammation and tissue synthesis stimulated by a pro-healing environment. Natural polymers have been increasingly studied for applications in health care due to their biocompatibility, biodegradability, and nontoxity (Mali et al., 2006). Topical application of honey to wound has been recognized for centuries as effective in controlling infection and producing a clean granulating wound bed. The recorded observations show that inflammation, swelling, and pain are quickly reduced, unpleasant odors cease, sloughing of necrotic tissue occurs without the need for debridement, dressings can be removed painlessly (Dunford et al., 2000; Molan, 1999). It is one of the most enduring materials to be used in wound care, attributed to its antibacterial, anti-inflammatory, and antioxidant properties (Khaled. M. A. Hussin et al., 2021). Honey is mildly acidic and has a pH between topical acidification of wounds promotes healing (Molan & Allen, 1996; Molan & Betts, 2004). The hydrogen peroxide produced by honey is responsible for the stimulation of tissue growth. Hydrogen peroxide has been shown to stimulate fibroblast growth in cell culture at micro- and nanomolar concentrations (Schmidt et al., 1993). The medical and nutritional properties of honey depend on its chemical composition. The chemical composition of honey varies depending on the plant source, season, and production methods (Moore et al., 2001). Honey has an obvious potential for use in a variety of clinical settings, and while a few clinics and individuals are using honey therapeutically, further research is needed to determine whether the source of honey can affect wound healing. The present paper discusses the effect of different types of honey for management of an full thickness skin wound in dogs.

MATERIALS AND METHODS

Study design:

A complete randomized design was used to determine the efficacy of Different Types of Honey on wound healing in dogs. The experimental protocol was approved by the Libyan national committee for biosafety and bioethics (Reference No: LNCBB05-2022).

Animals:

Twenty-four healthy female dogs weighing between 8 to 12 kg were used in this study. The animals were randomly into four experimental groups with each group consisted of 6 animals. Group I was left untreated and acts as control group. Group II, III and IV were treated with Sidr honey, Arbutus unedo honey and Eucalyptus honey respectively.The progress of wound healing was recorded at dyes 7, 14and Day 21 post-operation. An Elizabethan collar was applied to prevent self-mutilation of the surgical site.

Honey samples:

Three selected honeys which produced by bees from Al-Jabal Al-Akhdar Libya kept under different planting were used in this study the floral sources were Sidr honey from (Sidr) tree, Arbutus unedo honey from (Arbutus unedo) tree and Eucalyptus honey from (Eucalyptus) tree. The honeys were named according to their floral sources. all the honeys were supplied from Beekeepers Association Al-Jabal Al-Akhdar, Libya.

Surgical protocol:
Dogs were anaesthetized with an lntramascular (IM) injection of ketamine (5 mg kg) and Xylazine (2.0 mg kg) into the caudal thigh muscle (Faraj et al., 2022). When fully anaesthetized, the animals were positioned on their dorsal area which was prepared aseptically for the creation of an open wound (4x4 cm). The skin was disinfected with hibiscrub, containing 0.6% chlorohexidin then with 70% alcohol solution and with 2% iodine solution (Druecke et al., 2004). Using a sterile millimetre ruler and cotton tipped applicator dipped in sterile methylene blue, a 4 cm2 was drawn on the skin. A full thickness skin defect in which all tissue down to and including the panniculus muscle was excised using no. 15 scalpel blade. The wound was covered with honey and kept on the wound and protected with a bandage. The limb was immobilized using a PVC splint and bandage.

Assessment of the wound:
The progress of open wound healing was recorded at 0, 7, 14 and 21 days post-wounding. All the wounds were digitally photographed in the presence of a standard reference ntler.
The wound and its circumference were measured using the ON 3D Measure application. Where this was done by taking pictures of the wounds, and this application calculates the length, depth and width of the wound accurately.

Percentage of wound contraction was calculated by:

Step 1: Total wound on dayn = Total wound area dayn / original wound area day 0 x1 00.

Step 2: Wound on dayn %)= 100) =total wound on dayn as % of original.

Histopathological analysis:
The skin samples were taken for histopathological examination at day 7, 14 and 21 post-operations. The skin samples were fixed in 10% formalin solution and embedded in paraffin. Tissue sections of 4-5 mm thickness were cut, stained with Haematoxylin and Eosin (H&E) and examined under light microscope. Digital photomicrographs were captured at representative locations using a digital camera attached to a Nikon Eclipse FX-35DX microscope.

Statistical analysis:
Data are expressed as mean±Standard Deviation (SD). The statistical analysis of data was per-formed using 2-way ANOVA using the SPSS® Statistical package (SPSS, Version 20.0, Chicago, Illinois, USA). The effects with $p<0.05$ were considered statistically significant.

RESULTS

The percentage of wound contraction was better in group IV up to day 14 (58.78%), however it was best in group II on days 14 and 21. The difference between groups II, III and IV compared to group I was evident on day 7. The percentage of wound contraction improved from 57.84% (day 14) to almost 92.43% on day 21 in group II but group IV showed an improvement from 17.56% (day 7) to almost 82.67% on day 21 and group III showed an improvement from 16.09% (day 7) to almost 78.12% on day 21. Nevertheless, group II demonstrated better than average total wound healing throughout the trial (Table 1).

Table: (1). Percentage of wound contraction mean.

Days	Control Group I	Sidr Group II	Arbutus unedo Group III	Eucalyptus Group IV
0	0	0	0	0
7	15.90%	16.20.65%	16.09%	17.56%
14	40.90%	57.84%	42.65%	58.78%
21	68.93%	92.43%	78.12%	82.67%

Values with different superscripts within a row differed sigificantly at $p<0.05$

No unabsorbed remnants were noticed during the next application. In groups I, III and IV animals, the wound colour was red up to day 14 post-operation and pink in colour from day 21. Groups II showed bright beefy red colour up to day 14 and it were Pink from day 21. Malodour was observed up to day 14 post-operation in group I and II animals. In group III and IV mild malodour were observed up to day 7. Serous exudate was noticed up to day 14 in group I. Mild serous exudate was noticed up to day 7 post-operation in groups III and IV animals. No exudate was seen in group II animals throughout the study (Figure. 1, 2 and 3).

Figure: (1). Open wound on day 7 post-wounding: post-wounding: A=Control group I, B= Sider Group II, C= Arbutus unedo group III, D= Eucalyptus group IV.

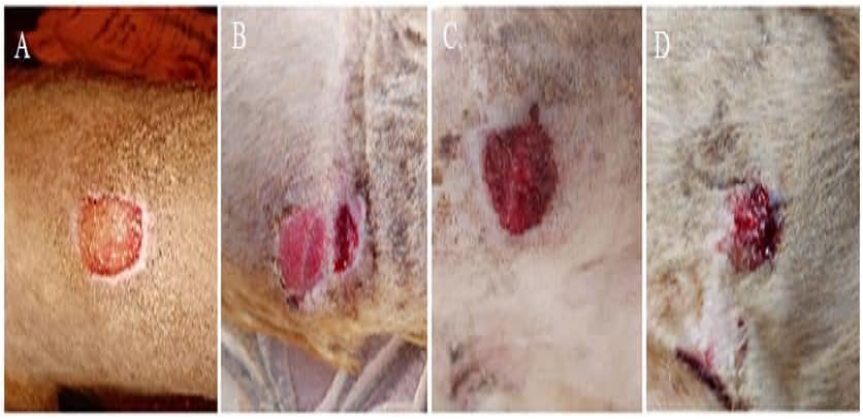

Figure: (2). Open wound on day 14 post-wounding: post-wounding: A=Control group I, B= Sider Group II, C= Arbutus unedo group III, D= Eucalyptus group IV.

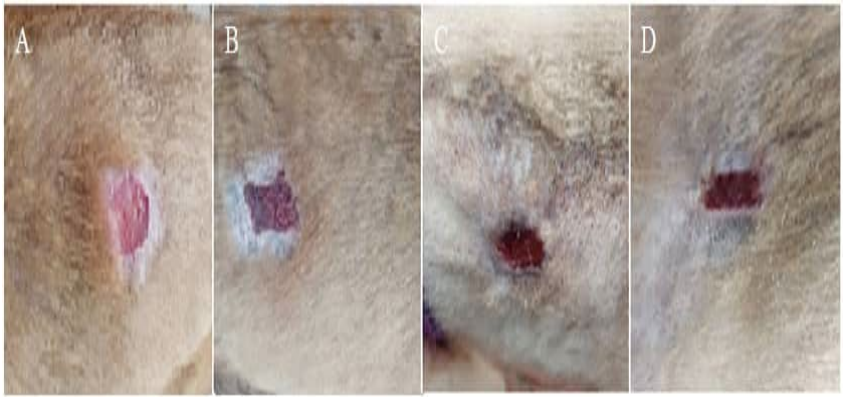

Figure: (3). Open wound on day 21 post-wounding: A=Control group I, B= Sider Group II, C= Arbutus unedo group III, D= Eucalyptus group IV.

Histopathological Study

After 21 days of injury, epidermal regeneration was observed in all experimental wounds. Histopathologic comparisons showed that on day 21, sidr hony treated wounds resulted in better re-epithelialization as compared to the control, Arbutus unedo hony and Eucalyptus hony treated dogs. In addition, the inflammatory cells were absent in all treated wounds. In the sidr hony, though new epithelium was noted to regenerate, inflammatory cells particularly neutrophils and macrophages were still present on the upper dermis. Less scab formation was seen in the wounds treated with Arbutus unedo hony, Eucalyptus hony and untreated control wounds (Fig. 4,5,6).

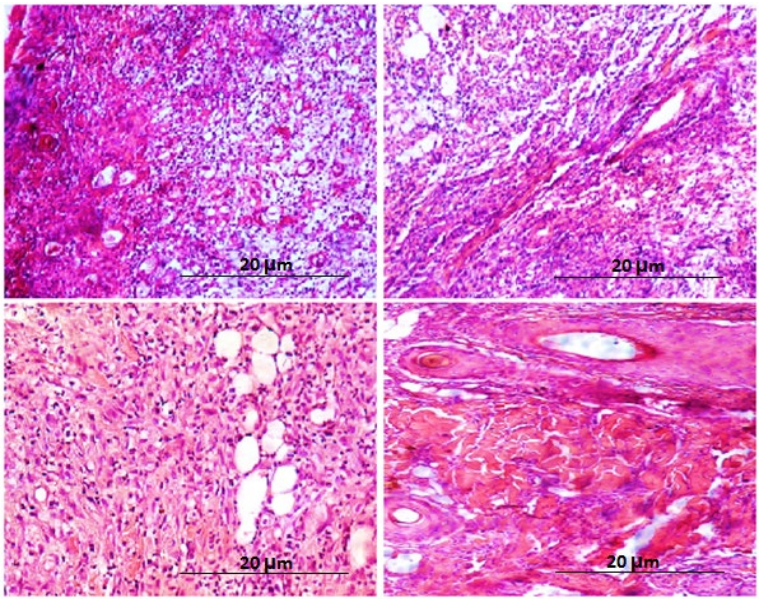

Figure: (4). Histopathology of the granulation tissue from the open wound day 7 post-wounding: A= group I, B= group II, C= group III, D= group IV. (H and E stain 40x); E = Endothelial cell; G = Granulation tissue; V = Blood Vessel.

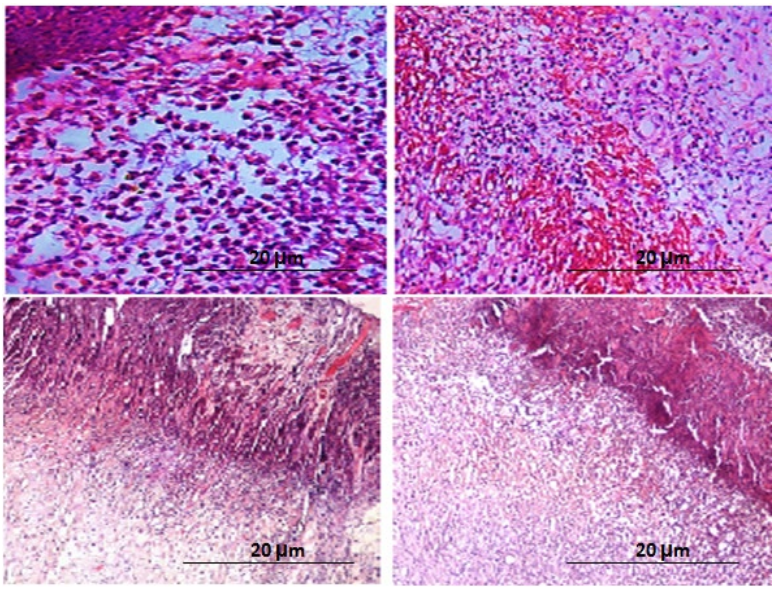

Figure: (5). Histopathology of the granulation tissue from the open wound day 14 post-wounding:A= group I, B= group II, C= group III, D= group IV. (H and E stain 40x); E = Endothelial cell; G = Granulation tissue; V = Blood Vessel.

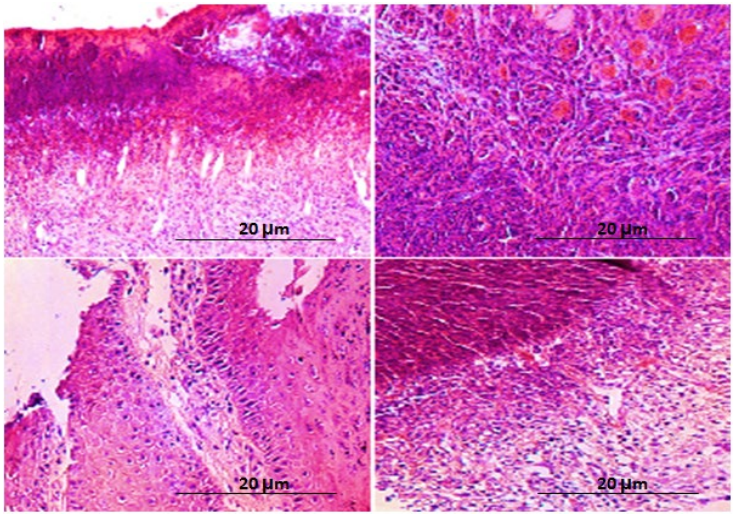

Figure: (6). Histopathology of the granulation tissue from the open wound day 21 post-wounding: A= group I, B= group II, C= group III, D= group IV. (H and E stain 40x); E = Endothelial cell; G = Granulation tissue; V = Blood Vessel.

DISCUSSION

In Groups II, III and IV, the application of honey was well tolerated by the animals. The honey is easy to apply on the wound without any adverse reaction and was well accepted by all the animals. The application of Sidr honey did not show any adhesion of the gauze during wound dressing. The colour of the wound bed in Group II was red while Groups I, III and IV showed a bright beefy red colour, which indicates healthy granulation tissue with neovascularisation, (James & Bayat, 2003) and resistance to infection until the epithelial barrier is re-established (Hosgood, 2003; Khaled et al., 2022; Pope, 1993).

The bright red colour observed is due to the micro vascular network throughout the granulation tissue (Tonnesen et al., 2000). The basic fibroblast growth factor set the stage for angiogenesis during the first three days of wound repair (Schäffer et al., 2004) and plays an important role in granulation tissue formation and the wound healing process (Takehara, 2000). Granulation in all the cases was flat without any exuberant nature; granulation tissue with a smooth surface facilitates migration of epithelial cells (Pope, 1993). On Days 21, the granulation tissue was observed to be pink in colour in all Groups, which indicates the final stage of wound healing. (James & Bayat, 2003). Mal-odour was observed up to Day 12 in Groups I; however, in Groups III and IV mild mal-odour was observed up to Day 7 which may be due to infection (James & Bayat, 2003). The presence of bacterial infection was the common cause for the mal-odour observed, because all the wounds were infected. Serous discharge was noticed up to Day 14 in Group I and mild serous discharge was noticed up to Day 7 in Groups III and IV; subsequently, the discharge was reduced because healthy vascular granulation tissue is resistant to infection (Hosgood, 2003; Pope, 1993).

Wound epithelialisation of Group II was consistently better than Group I, and also better then Groups III and IV because the reepithelialisation process in a full-thickness wound is accelerated by the presence of collagen and its proliferation is essential for optimal wound healing (Rangaraj et al., 2011). The percentage of wound contraction on post wound Days 7 of all Groups showed no significant difference, this may be because of an inherent property of fibroblasts that appears early in the process of wound contraction, which, after some time, do not contract as forcefully as those that appear later (Bohling et al., 2004b; Khaled. M. A et al., 2014). reported that (Schäffer et al., 2004) there is increased fibroblast activity of the body until post-wound Day 14. On Days 14 and 21,

Group II was significantly better than Groups I, III and IV since basic fibroblast growth factor effectively accelerated wound fibroblast proliferation (Kawai et al., 2000) and has a characteristic myofibroblastic appearance, which plays a critical role in closure and healing (Cheng et al., 1999).

The mean percentage of wound contraction of Group II on Day 21 was significantly better than other groups; in open wounds, contraction becomes an important feature and epithelialisation assumes a more predominant role. However, the two processes are independent of each other (Khaled et al., 2016; Probst, 2003). All the groups showed maximum rates of contraction in the period between Days 7 and 14 post-wound; similar findings have been previously reported (Aljady et al., 2000; Baie & Sheikh, 2000; Bohling et al., 2004b; Jothi et al., 2006). The percentage of total wound healing of Group II was significantly better then Group I and consistently better than Group III and IV because of more rapid epithelialisation; there is a corresponding reduction in area of exposed granulation tissue in the wound (Bohling et al., 2004b). On Day 20, among Groups II, III and IV in there was no significant difference but healing was significantly better than in Group I. Basic FGF is well known for promoting the proliferation of almost all cells associated with wound healing (Michiyo et al., 2005). All the groups showed maximum mean percentage of total wound healing from Days 7 to 14; this is in concurrence with the results of (Bohling et al., 2004a) but differs from the findings of (Swaim et al., 1993).

Sidr honey is a biocompatible protein that does not interfere with the body's normal immunologic response and can be used in non-healing chronic wounds, which require a trigger to stimulate the normal healing process.

CONCLUSION

Honey can be a better wound healing biomaterial in dogs. It can be used as a less expensive skin substitute in order to stimulate and promote wound healing in animals.

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ETHICS

The protocol for the research project and the animals experimental for research was approved by the Libyan National committee for Biosafety & Bioethics of the faculty of Veterinary Medicine, Omar Al-Mukhtar University (LNCBB-05-2022).

Duality of interest: The authors declare that they have no duality of interest associated with this manuscript.

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