Review of animal models used to study effects of bee products on wound healing: findings and applications

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Abstract

Non-healing wounds are associated with high morbidity and might greatly impact a patient’s well-being and economic status. For many years, scientific research has focused on developing and testing several natural and synthetic materials that enhance the rate of wound healing or eliminate healing complications. Honey has been used for thousands of years as a traditional remedy for many ailments. Recently, honey has reemerged as a promising wound care product especially for infected wounds and for wounds in diabetic patients. In addition to its proposed potent broad-spectrum antibacterial properties, honey has been claimed to promote wound healing by reducing wound hyperaemia, oedema, and exudate, and by stimulating angiogenesis, granulation tissue formation and epithelialisation. Several animal models, including large animals, dogs and cats, and different species of laboratory animals have been used to investigate the efficacy and safety of various natural and synthetic agents for wound healing enhancement. Interpreting the results obtained by these studies is, however, rather difficult and usually hampered by many limiting factors including great variation in types and origins of honey, the type of animal species used as models, the type of wounds, the number of animals, the number and type of controls, and variation in treatment protocols. In this article, we provide a comprehensive review of the most recent findings and applications of published experimental and clinical trials using honey as an agent for wound healing enhancement in different animal models.

Keywords: animal models, honey, wound healing.

Introduction

The emergence of antimicrobial-resistant bacteria is a great challenge facing both human and veterinary medicine today (22). In fact, research has been focusing in the last few decades on an alternative therapy that can potentially prevent and/or eliminate the threat of microbial resistance, enhance wound healing, and stimulate tissue repair. Honey is a well-balanced and nutritious food and has been considered as an effective remedy for many illnesses (7, 22, 27, 33). Unfortunately, there is no literature documenting the phytochemical properties of the types of honey used in studies on wound healing in both human and veterinary medicine. It is intuitive to assume that honey types vary in the properties of their ingredients. This variation could be attributed to many factors, including the floral source of honey as quite significant.

Recently, the superior medicinal properties of honey have prompted researchers to refocus their efforts on this substance. Various mechanisms are believed to contribute to the healing properties of honey including an anti-inflammatory effect, a pain-reducing effect, a deodorising effect, reduction of wound exudate, enhancement of the immune response, stimulation of auto-necrotic tissue debridement, mechanical protection of wound surface, stimulation of healthy granulation tissue formation, minimising of scar tissue formation, and reduction of the need for more invasive and expensive surgical techniques such as skin grafting (4, 20, 32, 35).
Animal models used to evaluate the effect of honey on wound healing

Cattle, sheep and goats

Honey has been used for the treatment of foot and mouth disease (FMD) lesions in dairy cattle in Kenya (15). Necrotic tissues were pre-cleansed with sodium bicarbonate solution 97% before daily application of raw honey or finger millet flour for three consecutive days. Animals which received such treatment were reported to resume feeding just a few days after the honey was applied and the lesions were completely healed. It was recommended to use such honey protocols in managing lesions associated with FMD in dairy cattle due to their availability, cheap prices, easy application, and rapid curative effect on necrotic lesions associated with the disease.

In Algeria, a fairly recent case report has described the use of locally-produced Euphorbia honey for wound care after eye enucleation in a dairy cow (23). Postoperatively, the wound was irrigated with normal saline before topical application of honey. During the first few days after surgery, honey was applied once or twice a day, then once daily for two weeks. Rapid formation of healthy granulation tissue and epithelialisation were observed without any apparent complications. The report has concluded that the use of Euphorbia honey enhances wound healing in trauma-induced wounds in cattle.

In Iraq, another study was conducted to evaluate the effect of honey on the healing of full-thickness wounds in Awassi sheep (5). The study showed faster wound healing (14–15 d) as evidenced histologically by granulation and collagen deposition, and lack of infection or abscess formation in the honey-treated group compared to the saline-matched control group. It was concluded that honey should be recommended for topical use on full-thickness wounds in Awassi sheep.

In Nigeria, blossom honey was successfully used for the treatment of surgically-created wounds in a goat model (2). In this study, wound epithelialisation and contraction was significantly faster in the honey-treated group.

Horses and donkeys

The effect of topical application of Manuka honey on second-intention wound healing in horses was assessed in a preliminary study conducted in the United States (10). Full-thickness wounds were surgically created on both metacarpals of eight horses. Wound contamination was induced by covering all wound surfaces with fresh horse faeces and bandaging for 12 h. Wounds on the first limb received daily treatment of Manuka honey, whereas wounds on the other limb were left untreated as the control. Researchers measured the wound area 1 d post wound creation, then weekly for eight weeks. Although Manuka honey enhanced the wound contraction rate and wounds appeared smaller at day 42 of the study, using this wound-healing model did not significantly affect the overall healing time of full-thickness distal limb wounds.

Experimentally, the efficacy of honey and cod liver oil mixture in the treatment of wounds was further experimentally evaluated using 18 circular wounds (4 cm radius) on the right metacarpus and left metatarsus in nine healthy donkeys (3). The animals were divided into three treatment groups. Wound surfaces located on metacarpi were treated using gauze soaked in honey, cod liver oil or honey/cod liver oil mixture respectively and compared with the untreated control wounds on the metatarsi. Thirty days post wound creation, complete wound healing had occurred, as evidenced by histological evaluation. The results of this study revealed a reduction in wound size after treatment with honey, cod liver oil, and their mixture. Histopathological evaluation revealed complete epithelialisation in wounds treated with either honey or cod liver oil and incomplete epithelialisation in wounds treated with honey/cod liver oil mixture, although a higher level of granulation tissue maturity and an increased number of fibroblasts were found in wounds treated with honey/cod liver oil mixture. The study recommended the use of honey/cod liver oil mixture for treatment of chronic and traumatised wounds, whereas the use of honey or cod liver oil alone was recommended for treatment of other wounds.

In Egypt, the beneficial effects of propolis as a wound dressing were investigated in 35 old wounds affecting the shoulders and necks of 18 horses and 14 donkeys (18). In addition, the study compared the therapeutic effects of propolis, honey, and normal saline dressings on 15 full-thickness wounds surgically created in the shoulder area of five healthy donkeys. After two-to-three weeks of treatment of the old wounds with propolis dressings, a marked reduction of wound size was noticed. In both clinical and histopathological evaluations, treatment of surgical wounds with propolis has shown advantageous outcomes compared to treatment with honey or normal saline. In conclusion, propolis was found to be more beneficial in the treatment of old equine wounds than honey.

The effects of topical application of pure Manuka honey or Manuka honey gel on second-intention healing of contaminated and non-contaminated distal forelimb wounds in horses were evaluated (9). Ten Standardbred horses were used and five full-thickness skin wounds (4 cm²) were created on both metacarpi. Contamination was induced by covering wounds on one forelimb with fresh horse faeces and bandaging for 24 h. Wounds on the contralateral forelimb were left uncontaminated. Wounds on both limbs were assigned into five treatment groups: Manuka honey (Unique Manuka Factor (UMF) 20) applied for 12 d; water-based gel applied for 12 d; UMF 20 honey gel applied for 12 d; UMF 20 honey gel applied until
complete healing; and the untreated control. Protective bandages were applied after application of each treatment. Bandages were applied daily for 12 d then removed to allow healing by the second intention. The time of complete wound healing was obtained by measuring the wound area (by digital photography and analysis of digital images using specialised software) at day 1 of the study and then once weekly until day 42. Results of this study showed that wounds treated with UMF 20 honey gel achieved the fastest healing rate. Surprisingly however, a faster rate of wound healing was noticed in the contaminated wounds than the non-contaminated wounds. The study concluded that the use of UMF 20 Manuka honey or UMF 20 honey gel reduces the overall time required for complete healing when compared to water-based gel-treated or untreated control wounds.

In a study evaluating the antimicrobial activity of 11 different types of honey against 10 isolates of common equine wound pathogens including methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* (11), it was revealed that eight types of honey inhibited all 10 isolates at variable concentrations (<2% to 16% v/v).

**Dogs and cats**

In a study conducted in Nigeria, researchers evaluated the effects of honey, glutamine, and honey-glutamine combination on the healing of small intestinal wounds following massive small intestinal resection in 24 young (three-four-month-old) mongrel dogs (13). The dogs underwent surgical resection of 70% of the small intestine. Then the dogs were assigned into four treatment groups and received oral glutamine, oral glutamine-honey combination, oral honey, or normal saline. Body weight was evaluated for 15 d after the beginning of treatments and intestinal biopsies were obtained before and after treatment and were subjected to morphometric and histological evaluations. The results of this study demonstrated that small intestinal adaptation occurred in all groups by the end of the study (day 28). The best adaptive effect was observed in the glutamine-honey treated group followed by the glutamine, honey, and control groups. Oral treatment with glutamine-honey combination was shown to yield the best overall healing effect marked by increased body weight gain and desirable intestinal changes. Those changes were intestinal mucosal growth and adaptation as evidenced by increased residual intestinal villi height and density, as well as increased intestinal crypt depth. More interestingly, glutamine showed better effects than honey with a significant increase in villi height and width, as well as crypt depth. The study demonstrated that treatment with the glutamine-honey combination is more therapeutically advantageous than treatment with either honey or glutamine alone, and this combination was recommended for better healing outcomes in patients with small bowel syndrome. Another clinical study was performed in Iran to evaluate the effects of West Azerbaijan natural honey on healing time in dogs (12). Thirty dogs were divided into two groups; clinical (10 dogs) and histopathological (20 dogs). Rectangular (2.5 cm × 5 cm) wounds were created on both sides of the thoracolumbar regions. Wounds on the right side were considered the control and were irrigated only with normal saline, whereas wounds on the left side were considered the treatment group. Honey (20 g) was applied topically once daily in the treatment group after irrigation of the wounds with normal saline. Both groups were evaluated clinically and histopathologically at days 7, 14, 21, and 28 post-operatively. Honey was found to accelerate wound healing with less exudate formation and/or infection. The size of the wound area in the honey-treated group decreased significantly compared to that in the control group. The histopathological evaluation revealed more rapid development of connective tissue as well as less neutrophilic chemotaxis in the honey-treated group than in the control groups, starting from week 2 onwards. The study indicated that the use of non-boiled West Azerbaijan honey for treatment of surgical wounds is potentially effective. The effectiveness of using propolis for treatment of surgical full-thickness skin wounds in dogs was evaluated in the study by Abdel-Wahed *et al.* (1). The study also compared the effects of propolis with honey-cod liver oil mixture, panthenol, and Moist Exposed Burn Ointment (Mebo). Five full-thickness wounds (2 cm × 3 cm) were created under aseptic conditions on the back area of 20 mongrel healthy dogs. Each wound was assigned a specific treatment; propolis powder, honey-cod liver oil mixture, panthenol, Mebo, and normal saline as a control. Treatments were applied topically under a bandage which was changed daily throughout the 1st week, every the 4th d for the following two weeks and then once a week until complete healing. Wounds were observed clinically and wound contraction percentage was calculated for each wound. Skin biopsies were obtained from all wounds at 1, 3, 6, and 16 d post wound creation for histological evaluation. The study indicated that treatment of wounds with propolis resulted in better clinical characteristics as well as marked reduction in wound size (increased wound contraction). Histologically, propolis treated wounds showed advantageous results compared to other treatments.

In Ontario, Canada, the successful use of honey was described as a wound dressing for the treatment of an open wound in a cat, which resulted from feline gangrenous mastitis (34). The wound was successfully managed by surgical debridement after lavage with diluted betadine solution and removal of necrotic tissue followed by topical application twice daily of natural non-pasteurised honey.
Laboratory animals (rats, mice, and rabbits)

To our knowledge, since 2010, at least 17 clinical trials and experimental control studies (Table 1) have been possible to cite, reporting evaluations using laboratory animals of the effects of various types of honey (14 studies) or honey products (3 studies) as agents for wound healing enhancement. Overall, careful review of the results of these studies reveals promising beneficial effects of honey or honey products on wound healing.

The use of propolis cream was found to provide the best overall wound healing effects in full-thickness skin wounds in rats (24). In this study, the inflammatory reaction was weaker in the propolis treated group than in the other groups treated with silver sulphudazaine (SSD) and bepanthane.

Unprocessed Teucrium polium honey was found to significantly accelerate the rate of wound healing as well as the wound tensile strength using a full-thickness wound model in rats (4).

The topical application of Tualang honey on full-thickness burn wounds contaminated with *P. aeruginosa* and *A. baumannii* resulted in the fastest rate of wound healing when compared with Hydrofiber silver– or Chitosan gel– treated wounds (31).

While oral zinc sulphate supplement was concluded to rather retard re-epithelialisation of full-thickness wounds in rats, the rate of healing was improved substantially when natural honey was added to the supplement and applied topically to the wound (30).

Mohd Zohdi *et al.* (27) demonstrated that sterile Gelam/hydrogel dressings displayed superior wound healing efficiency in treatment of deep partial-thickness burns when compared to Opsite and hydrogel dressings. The authors recommended using Gelam/hydrogel dressings as an effective alternative wound care product for the treatment of such types of burns. In another study, Gelam honey accelerated the healing of full-thickness excisional skin wounds with less scab formation in comparison to the controls (27).

In the study evaluating the healing effects of an ointment containing honey, fish oil, *Hypericum perforatum* L., and *Achillea millefolium* L. on full-thickness skin wounds, there was a significant improvement in the healing rates in treated wounds when compared to phenytin-treated wounds and untreated control wounds (25).

Other authors recommended the use of honey as a topical treatment of wounds in diabetic patients in both human and veterinary medicine (26). The study by Eyarefe *et al.* (13) revealed that both natural honey and amikacin enhanced wound healing in non-diabetic rat patients. Honey showed more promising results compared to amikacin in enhancing the healing of full-thickness punch biopsy wounds in diabetic rats (13).

In mice, the use of honey as a topical treatment of wounds was found to substantially accelerate wound healing with less inflammatory reaction in full-thickness skin wounds when compared to animal oil (sheep butter) and untreated controls (16).

Pure chestnut, pure rhododendron, and pure blossom honeys were suggested to help initiate wound healing by early elimination of the inflammatory reaction in full-thickness skin wounds in a rabbit model (29). In another study using rabbits, *Pistacia lentiscus* fatty oil (PLFO), and honey mixture was suggested to enhance healing of burn wounds as evidenced by faster contraction rates, especially when the mixture was applied during the inflammatory phase (26). It is worth mentioning that a standard burn wound model was developed in pigs to enable evaluation of histopathological parameters of wound healing under different circumstances (17).

Sidr honey was proven to produce superior wound healing effects to thyme and spring honeys in thermally- and chemically-induced burn wounds in rabbits (19). In this study, the effects of Sidr honey, thyme, and spring honey were compared to commercially available and synthetic burn healing agents such as Mebo and Fusidin and the results were comparable. Allwayzy (6) demonstrated accelerated wound healing as a result of topical application of honey/black seed oil mixture on experimental (ear punch) wounds. Moreover, topical application of natural honey and clay (mixture of montmorillonite) was found to accelerate wound healing and the mixture was suggested as an ideal dressing for excisional cutaneous wounds in rabbits (19). In a recent review, concerning the most topical wound healing products, the authors recommended the usage of Sidr honey for the treatment of different wounds in both human and animals (21).

Conclusions

Honey can be used as an alternative wound care remedy in different animal species because its effectiveness has been proven in various studies, and has been shown to vary depending on the plant source. When used properly, the use of honey is safe and does not involve complications.
**Table 1.** The use of laboratory animals as wound healing models for evaluation of honey as a wound treatment

<table>
<thead>
<tr>
<th>Type of honey</th>
<th>Comparative treatment and/or control</th>
<th>Animal species</th>
<th>Type of wound</th>
<th>Treatment protocol</th>
<th>Conclusions/mechanism of action</th>
<th>Ref. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure chestnut honey, pure rhododendron honey and pure blossom honey</td>
<td>Normal saline</td>
<td>Rabbits</td>
<td>Full-thickness skin wound</td>
<td>Every other day topical application of 0.5 mL of honey under bandage until complete epithelialisation</td>
<td>Although statistically not significant, honey-treated wounds showed increased granulation tissue formation, epithelialisation, angiogenesis, and fibroplasia in the early phases of healing</td>
<td>(29)</td>
</tr>
<tr>
<td>Propolis cream</td>
<td>Silver sulphurdiazine (SSD) and bepanthane</td>
<td>Rats</td>
<td>Full-thickness skin wound</td>
<td>Once per day application of 50% propolis cream for 14 d</td>
<td>Wounds treated with propolis were found in general to have a better wound healing effect with evidently less inflammatory reaction and proliferation of fibroblastic loose connective tissue</td>
<td>(24)</td>
</tr>
<tr>
<td>Natural honey</td>
<td>Animal oil (sheep butter or yellow oil) and untreated control</td>
<td>Mice</td>
<td>Full-thickness skin wound</td>
<td>Once per day topical application of 1 g of natural honey or animal oil for 10 d</td>
<td>Honey treated wounds showed decreased inflammation, oedema, and dehiscence and faster healing rate with faster formation of granulation tissue, and fibroplasias</td>
<td>(16)</td>
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<tr>
<td>Tualang honey (irradiated)</td>
<td>1% predisposition acetate, 0.3% ciprofloxacin, and ascorbic acid</td>
<td>Rabbits</td>
<td>Corneal alkali-induced injury</td>
<td>One drop of the honey (4 times daily) diluted in normal saline (30%) or oral administration of the honey (1.0 gm/kg/day) for 7 d</td>
<td>Tualang honey has equal wound healing effects compared to conventional treatments of alkali eye injuries</td>
<td>(8)</td>
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<td><em>Teucrium polium</em> honey (unprocessed)</td>
<td>Untreated control</td>
<td>Rats</td>
<td>Full-thickness skin wound</td>
<td>Twice per day topical application of 2 g of the compound until complete healing</td>
<td><em>Teucrium polium</em> honey promoted wound contraction, closure time and tensile strength</td>
<td>(4)</td>
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<tr>
<td>Local honey</td>
<td>Normal saline and untreated control</td>
<td>Rats</td>
<td>Laminectomy defect</td>
<td>Once 0.1 mL of honey applied at the laminectomy surgery site</td>
<td>Application of honey did not yield a significant reduction of peridural fibrosis</td>
<td>(14)</td>
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<tr>
<td>Tualang honey</td>
<td>Hydrofiber silver and Chitosan gel</td>
<td>Rats</td>
<td>Full-thickness skin burn contaminated with <em>P. aeruginosa</em> and <em>A. baumannii</em></td>
<td>Wound dressing using 0.2 mL of the honey changed every 3 d</td>
<td>Tualang honey resulted in a significant improvement in the healing of burn wounds</td>
<td>(31)</td>
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<tr>
<td>Natural honey</td>
<td>Zinc sulfate and untreated control</td>
<td>Rats</td>
<td>Full-thickness skin wound</td>
<td>Daily oral administration of dietary supplement containing honey and 36.3 mg of zinc sulfate and twice per day topical application of 10 mL of honey</td>
<td>Oral zinc sulfate alone may retard reepithelialisation, but, when used with natural honey (administered topically), it favourably influenced wound healing in non-zinc-deficient subjects</td>
<td>(30)</td>
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<tr>
<td>Sterile Gelam honey hydrogel dressing (Malaysian honey)</td>
<td>Control hydrogel dressing</td>
<td>Rats</td>
<td>Deep partial-thickness burns</td>
<td>Wound dressing using the honey hydrogel changed every 7 d until cessation of trial (day 28)</td>
<td>Application of honey hydrogel dressings significantly enhanced wound closure and accelerated the rate of re-epithelialisation with less inflammatory responses (27)</td>
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<td>Mixture of honey, fish oil, Hypericum perforatum L. and Achillea millefolium L.</td>
<td>Phenytoin and untreated control</td>
<td>Rats</td>
<td>Full-thickness skin wound</td>
<td>Once per day topical application of the mixture for 12 d</td>
<td>Treatment with the mixture significantly accelerated healing of the wounds (25)</td>
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<tr>
<td>Gelam honey (Malaysian honey)</td>
<td>Normal saline and untreated control</td>
<td>Rats</td>
<td>Full-thickness skin wounds</td>
<td>Once per day topical application</td>
<td>Gelam honey accelerate wound healing with less scab formation (32)</td>
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<tr>
<td>Local honey</td>
<td>Pistacia lentiscus fatty oil (PLFO) and Cicatryl</td>
<td>Rabbits</td>
<td>Burn wounds</td>
<td>Once daily topical application of 0.5 mL of PLFO, 0.5 g of honey, 0.5 g of mixture honey + PLFO (v/v), or 0.5 g of Cicatryl for 22 d</td>
<td>PLFO enhances the wound healing effect of honey when they are used as a mixture, especially during the inflammatory phase (26)</td>
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<tr>
<td>Sidr honey, thyme honey and spring honey</td>
<td>Mebo and Fusidin creams</td>
<td>Rabbits</td>
<td>Thermal and chemical induced burn wounds</td>
<td>Once per day application of either 500 mg of the honeys</td>
<td>Sidr honey produced superior wound healing effect in comparison to thyme honey or spring honey (19)</td>
<td></td>
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<tr>
<td>Local honey/black seed oil mixture</td>
<td>Untreated control</td>
<td>Rabbits</td>
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<td>Once per day application of the mixture for 28 d</td>
<td>Locally applied blackseed oil and honey mixture enhanced wound healing (6)</td>
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<td>Japanese honey, Acacia, Buckwheat flour, and Chinese milk vetch honey</td>
<td>Hydrocolloid dressing</td>
<td>Mice</td>
<td>Full-thickness skin wound</td>
<td>Once per day topical application of 0.1 mL of either Acacia honey, Buckwheat flour honey, or Chinese milk vetch honey</td>
<td>Japanese honey alone has limited benefit, although it reduces wound size in the inflammatory phase (28)</td>
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**References**


