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Current Medicinal Chemistry, 2018, *25*, 00-00

REVIEW ARTICLE

Honey: An Effective Regenerative Medicine Product in Wound Management

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ARTICLE HISTORY

Received: February 01, 2018
Revised: April 09, 2018
Accepted: April 10, 2018

DOI:
10.2174/0929867325666180510141824

Abstract: Honey has successfully been used in the treatment of a broad spectrum of injuries including burns and non-healing wounds. It acts as an antibacterial and anti-biofilm agent with anti/pro-inflammatory properties. However, besides these traditional properties, recent evidence suggests that honey is also an immunomodulator in wound healing and contains several bee and plant-derived components that may speed up wound healing and tissue regeneration process. Identifying their exact mechanism of action allows better understanding of honey healing properties and promotes its wider translation into clinical practice.

This review will discuss the physiological basis for the use of honey in wound management, its current clinical uses, as well as the potential role of honey bioactive compounds in dermal regenerative medicine and tissue re-modeling.

Keywords: Honey, wound repair, regenerative medicine, tissue.

1. INTRODUCTION

Honeybee products, especially honey, have been used in traditional medicine since ancient times for wide spectrum of skin disorders such as burns and wounds of different origin [1]. Honey has recently been investigated as a wound-healing remedy in modern medicine. Some honey types are already used in clinical practice. These types are called medical-grade honey. The most used FDA-approved medical-grade honey is Medihoney®, manuka honey derived from Manuka tree (*Leptospermum scoparium*). Manuka type honey forced the research to discover that different types of honey possess different antibacterial and are well-explored [2-5].

Revamil® honey is another medical grade honey used in clinical practice but with a different mechanism of action than manuka honey [6]. Moreover, honey possesses other activities that are crucial for its wound healing properties such as, anti-biofilm, immunomodulatory and anti/pro-inflammatory environment [7-9].

In fact, until the advent of antibiotics in the 1930 and 40s, honey dressings were part of normal wound care. Different causes have led to a new and renewed interest in honey as a therapeutic product. In particular, the misuse of antibiotics, the emergence of bacteria resistant to current drugs and new knowledge of honey proprieties provide the rationale for using honey as a broad-spectrum agent. Moreover, the current medical literature indicates that there may be benefits in treating burns or wounds with honey.

The aim of this review is to discuss the use of honey in modern wound care management, its current clinical uses, as well as the potential role of honey bioactive compounds in dermal regenerative medicine and tissue re-modeling, providing an up-to-date assessment of the reported benefits of using honey.

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0929-8673/18 $58.00+.00

Table 1. Biological properties of honey in wound healing.

Activity	Components	Refs.
Antibacterial	Sugars	[48]
	Hydrogen peroxide	[16,17]
	Unspecified non-peroxide components	[35,36]
	Methylglyoxal	[25]
	Defensin-1	[31]
	Polyphenols	[33]
Anti-biofilm	Defensin-1	[9]
	MGO	[41]
	Carbohydrates	[43, 44]
Anti/pro-inflammatory	Polyphenols (flavonoids)	[51,53,54]
Immunomodulatory	Defensin-1	[68]
	Major royal jelly protein 1	[55]
	5.8 kDa component	[57]
	Arabinogalactan proteins type II	[65]

1.1. Honey Composition

Honey is primarily a sugar-rich food source for bees. There are at least about 200 compounds found in honey, which consist of sugars, water, and other substances such as proteins (enzymes), organic acids, vitamins (*i.e.* vitamin B6, thiamine, niacin, riboflavin and pantothenic acid), minerals (including calcium, copper, *etc.*), pigments, phenolic compounds, a large variety of volatile compounds, and solid particles derived from harvesting [10, 11].

The chemical composition, color, aroma, and taste of honey depend on the botanical origin, geographical and climate areas and honeybee species involved, and are affected by several conditions, as weather, processing, manipulation, and storage [4, 12, 13].

2. ANTIBACTERIAL PROPERTIES OF HONEY

Currently, many researchers have reported the antibacterial activity of honey and found that natural honey has a broad-spectrum antibacterial activity when tested against pathogenic bacteria, oral bacteria as well as food spoilage bacteria. Honey has been reported to have an inhibitory effect on more than 60 species of bacteria including aerobes and anaerobes, Gram-positive and Gram-negative bacteria [14].

The high concentrations of sugars (about 80%) combined with less than 18% of water cause osmotic stress preventing spoilage of honey by microorganisms. The sugar content retains the antibacterial activity of honey also if diluted to 30-40% [15].

During the 1960s, hydrogen peroxide (H_2O_2) was recognized as a major antibacterial compound present in honey [16, 17].

Besides H_2O_2 produced in most types of honey by the endogenous enzyme glucose oxidase, several other factors have been found to be responsible for the antibacterial activities of honey [18].

In fact, several authors have demonstrated that honey may retain its antimicrobial activity even in the presence of catalase, and thus this type of honey is regarded as "non-peroxide honey" because their antibacterial properties are due to non-peroxide components [18-20].

2.1. Acidic Environment

Honey is characteristically acidic with pH between 3.2 and 4.5, which is low enough to be inhibitory to several bacterial pathogens [14]. The minimum pH values for growth of some common pathogenic species are: 4.3 for *E. coli*, 4.0 for *Salmonella* spp, 4.4 for *P. aeruginosa*, 4.5 for *S. pyogenes*. Thus, the acidity of honey is a significant antibacterial factor [14].

2.2. Peroxide Honey

The most known honey antimicrobial agent is H_2O_2, the concentration of which is determined by relative

levels of glucose oxidase, synthesized by the bee and catalase originating from flower pollen [18].

Most types of honey generate H_2O_2, by means of enzymatic activity of gluconic oxidase, added to the nectar by bees, that converts glucose into H_2O_2 and gluconic acid under aerobic conditions [21].

Some types of honey do not produce H_2O_2 at low levels, and several factors can affect this low production such as inactivation of glucose oxidase by exposure to heat or light [22, 23] or a direct inhibition by catalase originating from pollen, nectar or microorganisms [24].

2.3. Methylglyoxal

For various honey, there is a substantial non-peroxide antibacterial activity [19]. Manuka honey, produced from New Zealand Manuka tree *L. scoparium*, is known for the presence of non-peroxide antimicrobial components.

In manuka honey, there is a very high level of methylglyoxal (MGO) [25]. The high levels of MGO in manuka honey are due to the conversion of dihydroxyacetone (DHA) presented at very high concentrations in the nectar of *L. scoparium* flowers [26]. However, MGO also derives from sugars during heat treatment or prolonged storage of carbohydrate-containing foods and beverages [25].

The overall antibacterial activity of manuka honey is graded on one of the two scales: MGO concentration within the honey, or unique manuka factor (UMF). The UMF rating is based on a linear relationship with phenol when tested against *Staphylococcus aureus* [26, 27].

MGO is a major antibacterial factor of manuka honey, but it is interesting to note that its neutralization abolished the activity of manuka honey against *S. aureus* and substantially reduced the activity against *Bacillus subtilis* but did not affect the activity against *Escherichia coli* and *Pseudomonas aeruginosa* [28]. It suggests, thatother compounds (e.g. phytochemicals), and/or possible synergistic effects of these compounds/mechanisms present in honey might contribute to the overall antibacterial activity.

Roberts *et al.* (2014) showed that manuka honey inhibits the expression of flagella associated genes. Recently, it was discovered that MGO either directly damages or inhibits the formation of fimbriae and flagella [29, 30].

2.4. Bee defensin-1

First honeybee antibacterial peptide was isolated from royal jelly, therefore named royalisin [31]. Bee-derived antibacterial peptide was also identified in honey and designated as defensin-1 [2]. Later was discovered that royalisin and defensin-1 are products of the same polymorphic gene *defensin-1* [32].

Bee defensin-1 has potent activity but only against Gram-positive bacteria including *B. subtilis*, *S. aureus*, and *Paenibacillus larvae* [31]. Defensin-1 is an important compound in the wound-healing activity of honey. Besides antibacterial activity recombinant form of defensin-1 takes part in the anti-biofilm activity of natural honey against wound pathogens [9].

2.5. Other Antibacterial Components

Mundo *et al.* described that after a combination of H_2O_2 neutralization and degradation of proteinaceous compounds, several kinds of honey retain activity against *Bacillus stearothermophilis* but not against several other microorganisms including the highly MGO-susceptible *S. aureus* [33]. This observation proposes that the additional activity in these honey is exhibited by other components than H_2O_2, bee defensin-1, or MGO.

The role of some phenolic compounds originating from plants has been proposed as an important factor for the non-peroxide antibacterial activity of honey. Several antibacterial phenolic compounds have been identified in honey [34]. However, polyphenols are mainly responsible for honey anti-oxidant properties. Some studies suggest that phenolic contents are in correlation with antibacterial activity of honey [35, 36]. Honey phenolics/H_2O_2 – induced oxidative stress together with Maillard reaction-like products in honey underline the anti-oxidant and partially antibacterial activity [37].

3. ANTI-BIOFILM ACTIVITY OF HONEY IN WOUND MANAGEMENT

Cooperating bacteria forming a biofilm in wounds are a costly source of suffering. Biofilm protects living bacteria from patient's immune response. Honey is being widely tested as an anti-biofilm agent. Until now, a few mechanisms of honey anti-biofilm action were described. Manukau honey were extensively tested. Medihoney®, manuka-type medical grade honey, and Norwegian honey were found to be bactericidal against methicillin-resistant *S. aureus* (MRSA) and methicillin-resistant *Staphylococcus epidermidis* (MRSE), extended-spectrum beta-lactamase (ESBL) *Klebsiella pneumonia* and *P. aeruginosa* [38] and biofilm were

penetrated by a bactericidal compound of honey. In other studies, manuka honey showed *in vitro* bactericidal properties for *S. aureus* including MRSA strains (NCTC 8325, ATCC 25923, HA-MRSA, CA-MRSA), *P. aeruginosa*, *Streptococcus pyogenes* bacterial biofilms [39]. Manukatype honey can eradicate biofilms produced by *S. aureus* strains with different biofilm-forming abilities [40]. Manuka honey inhibits the development of *S. pyogenes* biofilms and reduces the expression of two fibronectin binding proteins.

Different mechanisms of anti-biofilm action of manukatype honey were described so far. MGO was thought to play the main role in the anti-biofilm activity of manukatype honey. Even though the effectiveness of different manukatype honey increases with MGO content, equivalent amounts of MGO did not prevent or eliminate biofilm and MGO requires other components for its anti-biofilm activity [41].

Manukatype honey disrupts the established *S. pyogenes* biofilm by specific interruption of binding to host tissue ligands and by inhibition fibronectin binding by a reduction in the expression of genes encoding two major fibronectin-binding streptococcal surface proteins [40].

Moreover, manuka-type honey down-regulates transcription of alpha-haemolysin, a protein that elicits host cells lysis, decreases transcription of the major structural flagellin protein thereby resulting in limited motility *in vitro* [29, 42]. Besides manuka honey, different types of honey showed other anti-biofilm mechanisms. Some authors suggest that carbohydrates in honey are the main components that affect quorum sensing [43, 44]. No difference was shown between 4 different kinds of honey (manuka honey, honeydew, acacia, hawthorn) on wound isolates *Proteus mirabilis* and *Enterobacter cloacae* biofilm formation [45]. However, manuka honey showed the most potent anti-biofilm activity. Moreover, MGO was able to diffuse through the established *P. mirabilis* biofilm matrix and kill bacteria [45]. Recently, it was discovered that defensin-1 presented in every honey takes part in the anti-biofilm activity where its recombinant form could prevent biofilm development either by interfering in bactericidal adhesion to a surface or by inhibiting the growth of attached cells in the early stages of biofilm formation [9]. Honey is also suggested as a potential adjunct treatment with rifampicin for chronic wounds infected with staphylococcal biofilms [7].

4. HONEY AND WOUND HEALING MECHANISMS

A healthy environment is normally required to maintain and/or sustain a correct and physiological wound repair. One of the strategies to keep the healing process ongoing is to protect damaged tissue form any microbial infection. Honey exhibits antibacterial, anti-biofilm, anti-oxidant, anti/pro-inflammatory and immunomodulatory properties that altogether create an ideal wound-healing remedy.

A wound is a disruption (from violence, accident, or surgery) of the continuity of tissue integrity, damaging also neighboring areas.

The efficacy of honey as agent for wound healing is well established. It can promote wound healing and tissue repair processes with little or no formation of scars [46, 47].

In spite of a vast amount of literature reporting the clinical efficacy of honey in wound healing, the underlying mechanism of its action are still largely obscure.

4.1. Physicochemical Properties

The physicochemical properties (e.g. low pH and high viscosity) of honey can contribute not only to its antibacterial properties but also to its wound-healing properties.

The physical properties of honey make it an ideal moist wound dressing. In particular, the viscosity of honey (that can vary from floral to floral source) provides a protective barrier to prevent injuries from becoming infected sealing the wounds.

The sugar content of honey, and in particular glucose and fructose, can improve the local nutrition in the wound and promotes rapid epithelialization.

Honey also helps in the deodorization of infected wounds, by providing an alternative to the amino acids from the serum and dead cells that are metabolized by bacteria [48].

Some authors have also proposed that the acidic pH of honey may help to create and maintain optimal conditions for fibroblasts, which require mildly acidic environment for fibroblastic activities such as migration, proliferation, and organization of collagen [49].

4.2. Hydrogen Peroxide

H_2O_2 can be a very harmful compound for cells and tissues. Honey continuously generates H_2O_2 at concentrations that inhibit bacterial growth. H_2O_2 at physiological concentrations may act as "messenger" modulating several cell signaling pathways involved in wound healing [50].

4.3. Anti-inflammatory / Pro-inflammatory Activity

Inflammation is one of the crucial stages of wound healing process. The anti-inflammatory effect of honey may be explained by several mechanisms of action: (i) inhibition of the classical complement pathway [51], (ii) inhibition of reactive oxidative species (ROS) formation [51], (iii) inhibition of leukocyte infiltration [52], (iv) inhibition of cyclo-oxygenase-2 (COX-2) and inducible NO synthase expression (iNOS) [53], (v) inhibition of tumor necrosis factor-alpha (TNF-α)-induced matrix metalloproteinase-9 (MMP-9) and cell growth-promotion in chronic wound fluids in human keratinocytes [54], (vi) up-regulation of matrix metalloproteinase-9 (MMP-9) mRNA expression in primary keratinocytes [55], (vii) decrease in collagen type IV in the basement membrane [55], (vii) reducing inflammatory pain involving the autonomic receptors [56].

On the contrary, honey also stimulates the release of pro-inflammatory cytokines (TNF-α, IL-1, IL-6) in human peripheral blood monocytes [57, 58]. Therefore, honey exhibits both anti-inflammatory and pro-inflammatory properties. It seems that honey acts according to the wound environment.

4.4. Anti-oxidative Activity

Chronic wounds are considered a highly oxidizing environment releasing ROS from infiltrating neutrophils and macrophages. ROS is thought to have antibacterial properties against invading bacteria [59] but prolonged exposure to elevated levels of ROS causes cell damage and may inhibit healing process of both acute and chronic wounds. Various studies have shown that phenolic compounds are responsible for antioxidant activities of honey [36, 44, 60]. Additionally, changes in total phenolic content of melanoidins in heated versus unheated honey are strongly correlated with the changes in the antioxidant activity [61].

4.5. Immunomodulatory Activity

Antibacterial, anti-biofilm and anti/pro-inflammatory activities of honey are important attributes for wound healing agent. However, honey may also possess plant or bee-derived compounds with immunomodulatory effects, which can initiate or accelerate wound-healing process. Ranzato and co-workers have described that honey treatments showed very low cytotoxic effects on keratinocytes and fibroblasts, the main cell types of skin. They demonstrated *in vitro* that honey can be classified as a non-toxic substance, and used safely for external applications on healthy skin and as a dressing on wounds. In addition, scratch wound data and cell migration assays showed that honey induces a marked increase of the wound repair capabilities of keratinocytes and fibroblasts [62, 63].

Cutaneous wound healing restores the epidermal barrier against external environment during a process called re-epithelialization. A key feature of re-epithelialization is the migration of cells under the stimulus of injury signals, in a process called epithelial-mesenchymal transition (EMT). This EMT is a reversible change of cell phenotype, during which epithelial cells loosen cell-cell adhesion structures including adherens junctions and desmosomes, modulate their polarity and rearrange their cytoskeleton.

Ranzato *et al.* [63] demonstrated that during honey treatment, keratinocytes induced significant changes in the expression of EMT-regulatory genes, with differences according to types of honey utilized. In the same work, they also revealed the occurrence, in terms of cellular mechanisms, of different mechanisms demonstrated by the use of calcium and kinase inhibitors. These observations suggested the complexity of responses induced at cellular and molecular levels by the exposure to different kinds of honey.

A study of Majtan *et al.* [55] demonstrated that keratinocytes are responsible for an increase in the production of some cytokines (TNF-α, IL-1β and TGF-β) involved during inflammation and proliferative phase as well as after honey exposure there is an increase of MMP-9 and degradation of type IV collagen in the basement membrane. Tonks *et al.* [57] proposed that isolated 5.8 kDa component from manuka honey is responsible for the induction of TNF-α in human monocytes via Toll-like receptor (TLR) 4 [64]. Major royal jelly protein (MRJP) 1 was also associated with stimulation of TNF-α mRNA expression [55]. Another immunostimulatory component in honey belongs to the type II arabinogalactan proteins (110 kDa). They are able to induce the release of TNF-α from monocytic cell lines THP-1adn U937 [65]. Epithelization phase involves migration and proliferation of keratinocytes to cover the closing wound.

Ranzato *et al.* [63] later observed that various types of honey significantly stimulate MMP-9 expression, confirming that this MMP plays a pivotal role in keratinocyte wound healing boosted by honey. This study showed an increase in re-epithelialization rates and chemoattractant effects in presence of 0.1% (v/v) dilution of different honey. Also focal adhesion kinase (FAK) and rasGAP SH3 binding protein 1, which are involved in focal complex formation and cell locomotion, were activated by different honey types [63].

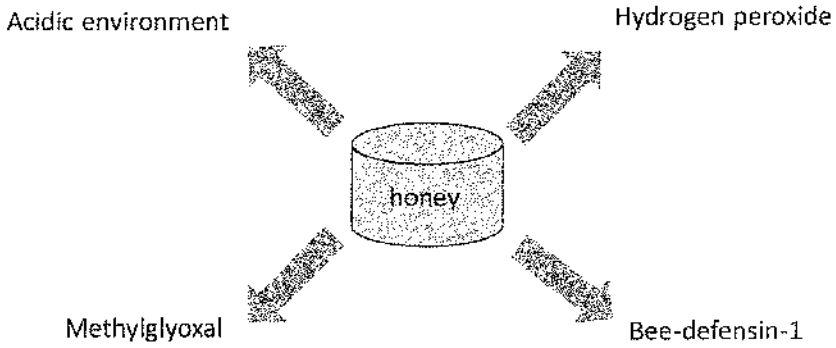

Fig. (1).

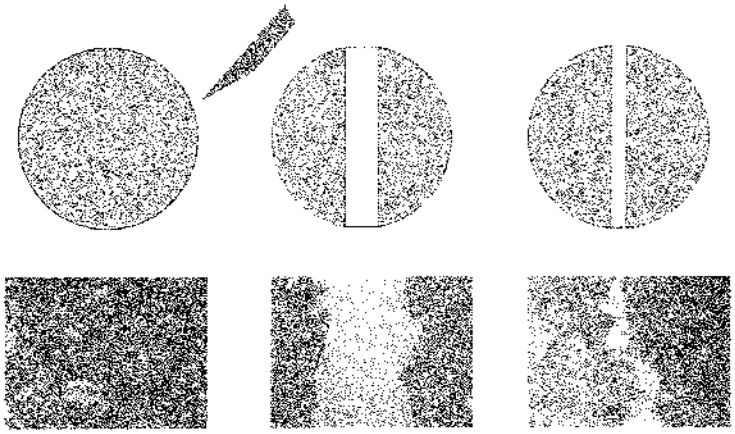

Fig. (2).

Honey dilution of 0.1% showed more potential for hypoxic wound healing compared to the normoxia marker conditions, considering cell migration, proliferation and reduced expression of hypoxic marker Hif 1α [66]. Dilution of 0.1% proved to have better biological potential to facilitate epithelial cell proliferation as well as migration both essential for wound healing [66].

Ahmed and co-workers outline the inhibitory effect of honey on platelet aggregation and blood coagulation [67].

Other findings provide a clear evidence that honey may act as a natural treatment for dermatological problems. Martinotti *et al.* [62] studied the biological activities on skin cells (fibroblasts and keratinocytes) after honeydew honey exposure.

Taken together, data coming from honeydew honey and findings on blossom honey indicate that honey are very active in the induction of wound repair mechanisms, thus supporting a bulk of the anecdotal and scientific evidence. Bucekova and co-workers [68] have demonstrated that defensin-1, an antibacterial peptide present in royal jelly and honey [69] is able to stimulate MMP-9 secretion keratinocytes and to induce keratinocyte migration and wound closure *in vitro*. Moreover, defensin-1 promoted re-epithelisation and wound closure in uninfected excision wounds. These data have suggested that defensin-1contributes to cutaneous wound closure by enhancing keratinocyte migration and MMP-9 secretion.

5. CLINICAL TRIALS

Several clinical trials have been conducted using honey for a broad spectrum of injuries (burns, ulcers and post-surgical wounds). However, not all types of honey exhibit equal antimicrobial potency and only a few meet the criteria for clinical usage. Honey must be sterilized by gamma radiation before application on the wound to inactivate spores of *Clostridium botulinum*. Gamma irradiation does not affect honey beneficial properties [70].

Manuka honey is the most frequently studied honey in human clinical trials claimed to have therapeutical advantages over other honey types. In recent years, many clinical trials used honey as a medical device to

treat various wound types such as burns, diabetic foot and venous ulcers, malignant, post-surgical and eyelid wounds. Manuka honey was applied in different forms either as 100% honey or incorporated in the matrix of wound dressings (hydrogels and alginate) [71-73].

Furthermore, local honey from different world regions are being tested for clinical treatment as well [74, 75].

Efem [76] conducted one of the first clinical trials of honey (floral source not specified) as a wound dressing, where 59 patients suffered from recalcitrant wounds and ulcers of different etiology. The study showed that honey debrided wounds rapidly, replacing gangrenous and necrotic tissue with granulation tissue and advancing epithelialization.

Subrahmanyam [77] conducted prospective randomized clinical trials investigating the effectiveness of honey as a topical agent for burns compared with other traditional remedies, and current pharmacologic and surgical methods usin gsilver sulfadiazine [78]. He found that the wounds treated with honey were characterized by healthy granulation tissue after an average of 6.8 days, with the majority completely healed by 10.4 days. The antibacterial activity of honey prevented wounds from secondary infections. It was concluded that honey dressings for burns were non-irritating, non-toxic, easily applicable and cheap.

Honey, and in particular manuka honey, has been used to treat animals with surgical or accidental wounds, particularly horses, with positive outcomes [79, 80].

However, despite the evidence from numerous *in vitro* and *in vivo* models that honey kills problematic wound pathogens, there is a paucity of robust clinical data for honey due to technical difficulties in performing a double-blind placebo-controlled trial on a distinctive substance like honey, ethical considerations, lack of interest by clinical practitioners and cost-versus-benefit to honey companies [81].

CONCLUSION

It has been showed that honey meets all criteria to be a suitable and effective remedy for wound treatment. Besides its well-described antibacterial, anti-biofilm and anti/pro-inflammatory properties, honey also exhibits potent immunomodulatory properties. The successful application of honey in the treatment of wound infections promotes other therapeutic uses. However, clinical evidence supporting the beneficial properties of honey is limited. We advocate randomized control trials and future research, that will elucidate the synergistic effect among biological active molecules in honey.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

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PMID: 29745320