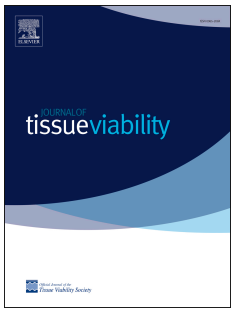
Accepted Manuscript



Biological Properties and Therapeutic Activities of Honey in Wound Healing: A narrative review and meta-analysis

Ahmad Oryan, D.V.M, Ph.D., Professor, Esmat Alemzadeh, Ali Moshiri

PII: S0965-206X(15)00097-2

DOI: 10.1016/j.jtv.2015.12.002

Reference: JTV 182

To appear in: *Journal of Tissue Viability*

Received Date: 10 March 2015

Accepted Date: 9 December 2015

Please cite this article as: Ahmad Oryan Alemzadeh E, Moshiri A, Biological Properties and Therapeutic Activities of Honey in Wound Healing: A narrative review and meta-analysis, *Journal of Tissue Viability* (2016), doi: 10.1016/j.jtv.2015.12.002.

Biological Properties and Therapeutic Activities of Honey in Wound Healing: A narrative review and meta-analysis

Running title: Honey & wound healing

Ahmad Oryan, Esmat Alemzadeh, Ali Moshiri

1) Department of Pathology, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

2) Department of Biotechnology, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

3) RAZI Drug Research Center, Iran University of Medical Sciences, Tehran, Iran

Correspondence to: Professor Ahmad Oryan, D.V.M, Ph.D.

E-mail address: oryan@shirazu.ac.ir

Biological Properties and Therapeutic Activities of Honey in Wound Healing: A narrative review and meta-analysis

Running title: Honey & wound healing

Abstract

For thousands of years, honey has been used for medicinal applications. The beneficial effects of honey, particularly its anti-microbial activity represent it as a useful option for management of various wounds. Honey contains major amounts of carbohydrates, lipids, amino acids, proteins, vitamin and minerals that have important roles in wound healing with minimum trauma during redressing. Because bees have different nutritional behavior and collect the nourishments from different and various plants, the produced honeys have different compositions. Thus different types of honey have different medicinal value leading to different effects on wound healing. This review clarifies the mechanisms and therapeutic properties of honey on wound healing. The mechanisms of action of honey in wound healing are majorly due to its hydrogen peroxide, high osmolality, acidity, non-peroxide factors, nitric oxide and phenols. Laboratory studies and clinical trials have shown that honey promotes autolytic debridement, stimulates growth of wound tissues and stimulates anti-inflammatory activities thus accelerates the wound healing processes. Compared with topical agents such as hydrofiber silver or silver sulfadiazine, honey is more effective in elimination of microbial contamination, reduction of wound area, promotion of re-epithelialization. In addition, honey improves the outcome of the wound healing by reducing the incidence and excessive scar formation. Therefore, application of honey can be an effective and economical approach in managing large and complicated wounds.

Key words: Honey; Hypertrophic scars; Medicinal wound healing

1. History and Background

Throughout history, honey has been well used for medicinal applications. Islamic traditions, ancient Egyptians, ancient Greek, Chinese and other countries and nations have used honey as a remedy for every illness [1]. In modern history and in World War I, honey was used for repair of battle wounds. For the first time in 1892, antimicrobial properties of honey were noted by Van Ketel. The role of honey in healing of infected wounds was first reported in Europe and USA in the mid-20th century [2,3]. However in the last decades and by development, introduction and wide application of modern antibiotics, the application of honey was reduced in a modern medicine. Advent antibiotic-resistant bacteria and the potent activity of honey against them renewed interest for its medical application [3]. Since then, many studies have shown that honey has several medicinal properties. For instance, honey is an anti-microbial agent, promotes autolytic debridement, stimulates growth of wound tissues to hasten healing and healing process in dormant wounds and finally initiates anti-inflammatory activity that rapidly reduces pain, edema and exudate production [4,5].

In this review, an overview of the current knowledge on the healing properties of honey has been illustrated and the mechanisms of action and therapeutic properties of honey in wound healing have been discussed in detail. In addition, the most recent and valuable clinical trial studies used honey for treating various wounds have been reviewed and discussed in order to find out whether honey is a valuable option for wound healing, modeling and remodeling.

2. Therapeutic properties of honey

Honey, due to its antimicrobial, anti-inflammatory and antioxidant activities, boosting effect on immune system, debridement action and stimulating role in wound regeneration, significantly

contributes in wound healing processes (Fig. 1, Table 1). Bellow, these beneficial biological properties have been discussed in detail.

2.1. Antimicrobial activity

The use of substances produced by honey bees (*Apis mellifera*), including propolis, honey, wax and venom in wound management has a long history which belongs to many centuries ago [6,7, 8]. When the body's immune response is unable to clear infection, antimicrobial therapy is considered [9]. Silver dressing has been used for managing wound infections particularly in the burn wounds because of its antibacterial properties; however components such as silver sulfadiazine may delay wound healing [10]. So far, many compounds have been evaluated for their antibacterial properties [11,12,13]. It is believed that honey can be used as a suitable alternative option for managing various infected wounds [14,15]. However, it should be kept in mind that bees with nutrition of various plants create different types of honeys [1,16]. The compositional differences of honeys can influence their medicinal value. Brudzynski et al. [17] analyzed bactericidal activity of eight honeys (six varieties of buckwheat, blueberry and manuka honeys). They suggested that the dose of honey is important in its bactericidal efficacy and the maximal bactericidal effect occurs with degradation of bacterial DNA. The manuka and buckwheat honey showed bactericidal effect at the range of concentrations between 6.25% to 50% v/v, while bactericidal effect was observed only at the highest concentration tested (50% v/v) for blueberry honey. Finally, they concluded that honeys of buckwheat origin, dose dependently possess powerful bactericidal effect [17].

In the recent years, much more interests are seen for evaluating the effects of honey on the antibiotic-resistant organisms. Indiscriminate use of antibiotics and advent resistant strains has a considerable effect on patient's morbidity and mortality [16,18,19]. The emergence of antibiotic-

resistant microbial strains can be an alarm that wound infections may once again become uncontrollable. Honey is a realistic antimicrobial in the treatment of infections. Theoretically, the increased use of honey can promote honey resistance, but to date, no honey resistant microbial strain has emerged and a possible explanation for this might be due to the multifactorial nature of the antimicrobial properties of honey that affects more than one target site [20,21,22]. Compared with other antimicrobial agents, honey produced a unique expression signature on the gene expression of *E.coli*. Most of the genes upregulated by Medihoney, are involved in stress responses and the majority of downregulated genes encoded for products, are involved in protein synthesis. These findings confirm that honey works by a different mode of action than the other conventional antibiotics [23].

It has been shown that biofilm biomass that was treated with manuka honey increased bacterial resistance, which is possibly due to the appearance of small colony variants within the microbial population [24]. In this regard, Cooper et al. [25] explained that although susceptibilities to manuka honey reduced during long-term stepwise resistance training, these changes were not permanent and honey-resistant mutants were not detected. They suggested that the risk of bacteria acquiring resistance to honey will be low if high concentrations are maintained clinically. High levels of antimicrobial agents can effectively inhibit viable bacteria before resistant strains emerge. Unlike some of the biocides, high amount of manuka honey is not cytotoxic [25].

Bacterial cell wall plays a fundamental role in maintaining the cell shape and sustaining bacterial growth. By inhibition of the cell wall synthesis, honey is able to induce structural changes in the bacterial cell wall. In a comparative study, it has been shown that honey similar to ampicillin changes the cell shape and outer lipopolysaccharide (LPS) membrane of *E. coli*. Since cell lyses

requires both cell wall and LPS disintegration, honey can be considered as a proper substance in antibacterial activities [26].

It is now accepted that bacterial populations in chronic wounds can arrange into biofilms. It has been shown that honey can affect on bacterial bioflms [27,28,29,30]. For instance, methylglyoxal (MGO) is an active ingredient of manuka honey which is an effective antimicrobial agent against planktonic and biofilms [31,32,33]. The high concentration MGO is responsible for antibacterial properties of honey [32,33]. MGO attacks the arginine residues in collagen and leads to disruption of collagen that promotes fibrosis in chronic tissue infections. Also, MGO by altering the structure and function of many important immunological proteins and enzymes is able to reduce the efficiency of peripheral blood immune-cell response [33]. Other honey components, such as glucose, fructose and a wide range of phytochemicals, also have an important role in reducing bacterial biofilm formation [27].

Many reports have confirmed antifungal activity of honey. Honey, not only inhibits fungal growth but also it reduces fungal toxin production [34,35,36]. Canonico et al. [35] suggested that the honey flavonoids can be potential alternatives to anticandidal agents. Honey flavonoids are able to attach to the yeast cell wall and perform their disruptive effects. In addition, the activity of honey is due to the changes it makes in the cell cycle progress, membrane integrity, mitochondrial function and biogenesis [35].

2.1.1. Mechanisms of anti-microbial activity of honey

Al-Waili et al. [1] classified these mechanisms into direct and indirect groups. Direct action is based on direct elimination of bacteria by honey components, and indirect action is antibacterial response of the whole organism toward bacteria. Factors that directly affect the bacteria and are toxic to pathogens include hydrogen peroxide factors, high osmolality, acidity, non-peroxide

factors, and phenols. Indirect antimicrobial actions include lymphocyte and antibody production, cytokines and immunomodulation, and nitric oxide.

2.1.1.1. Hydrogen peroxide

Hydrogen peroxide (H_2O_2) is commonly used to disinfect and sanitize medical equipment in hospital and reacts with the myeloperoxidase near bacteria within phagocytic vacuoles [1,9]. Hydrogen peroxide is one of the main components of honey that has an effective role in antimicrobial activity and is slowly released from honey due to interaction of the wound exudates with the honey's inherent glucose oxidase [3,37,38]. Glucose oxidase is added to nectar by bees and is able to convert glucose into hydrogen peroxide and gluconic acid under aerobic condition [3,14,39]. It has been shown that lower rates of hydrogen peroxide exist in more concentrated honeys and maximum accumulation of hydrogen peroxide occurs in honey concentrations of 30%–50% (v/v). Hence, lower concentrated honeys are more suitable in wound healing than the more concentrated ones [40]. More importantly, when honey is diluted, it is able to eliminate the antibiotic-resistant pathogens [41,42]. During ripening of honey, glucose oxidase is inactivated but it regains its activity on dilution of honey [40]. The reason of this incidence is unknown as yet; however, the previous studies indicated that it is not associated to constituents of honey but it may be the result of the low pH of full strength honey [43,44]. Many factors such as inactivation of glucose oxidase by heat, light and MGO, chemical scavenging of H_2O_2, destruction of H_2O_2 by catalase occurring in nectar and pollen and auto-oxidation of polyphenols/flavonoids can inactive hydrogen peroxide in honey [38,45,46,47]. These factors contribute to variable activities of some honeys under different conditions [48]. It has been shown that glucose oxidase remains stable at a temperature of up to 55 ^{0}C, while at 55-70 ^{0}C it declines [49]. Catalase which presents in flower pollens and in the body tissues, can destroy the

peroxide activity [3,37]. Benzoic acid, one of the compounds exists in honey, can react with hydrogen peroxide and create peroxy acids which are more stable and powerful antimicrobial agent than hydrogen peroxide. These acids are also stable in the presence of catalase [9]. Some types of honey may maintain their antimicrobial activities in the presence of catalase and thus are called “non-peroxide” honeys. Several components such as methylsyringate and MGO contribute to non-peroxide activity [9,37]. Although MGO is considered as a strong antimicrobial agent in manuka honey, recently it was found that MGO could also have negative effects on the structure and function of glucose oxidase and generation of hydrogen peroxide. MGO-treated honeys generate significantly lower amounts of H_2O_2 due to high reactivity of MGO with the glucose oxidase enzyme. This reaction forms high molecular weight adducts that decreases enzymatic activity of glucose oxidase and might be responsible for suppressing H_2O_2 generation in manuka honey [47,50]. Recent evidence is a possible explanation that why some researchers have failed to detect H_2O_2 as an antimicrobial component in manuka honey [51,52]. On the other hand, the antibacterial activity of honey, which greatly depends on the accumulation of hydrogen peroxide and reduction in the concentration of glucose oxidase, can significantly decrease hydrogen peroxide content [53]. Overall, it should be considered that the levels of hydrogen peroxide may significantly differ in honeys related to a region.

The presence of ascorbic acid in honey can promote the bactericidal activity of hydrogen peroxide. In gram-negative bacteria, a mixture of hydrogen peroxide and ascorbic acid increases the lysis and death of organism by lysozyme [1]. It has been shown that hydrogen peroxide can recruit leukocytes to wounds through a concentration gradient mechanism. Due to an oxidant induct, macrophages release vascular endothelial growth factor (VEGF) and stimulate angiogenesis. In acute inflammatory phase that neutrophils release bactericidal reactive oxygen

species, H_2O_2 is able to kill bacteria and prevent infection [1,37]. The use of hydrogen peroxide is typically not favorable for wound healing because it induces excessive inflammation and tissue damage. However, honey by disabling the free iron, which catalyses formation of the oxygen free radicals produced by hydrogen peroxide, reduces harmful effects of hydrogen peroxide. On the other hand, level of hydrogen peroxide in honey is too low so that its concentration is about 1000 times less than in the 3% solution commonly used as an antiseptic agent [54]. Interestingly, honey due to its high levels of antioxidants, can protect wound tissues from oxygen radicals produced by H_2O_2 [9]. In addition to antimicrobial activity, hydrogen peroxide stimulates fibroblast proliferation and angiogenesis and enhances cutaneous blood recruitment in ischemic ulcers [55,56].

2.1.1.2. Osmolarity

High osmolarity is considered as a valuable tool which limits bacterial growth and proliferation [1,9,38,57,58,59]. High osmolar solutions such as honey, glucose, and sugar pastes tie up water molecules [6]. Honey as a viscous fluid provides a protective barrier and prevent cross-infection of wounds [60]. When honey is topically applied over a wound, due to its high osmolarity, bacteria have insufficient accessibility to water for growing [6,57,61]. Consequently, the microorganisms become dehydrated and eventually die [42,61]. Also, osmotic pressure from honey draws out lymphatic fluid from the subcutaneous tissue to wound surface, which aids in removal of debris, necrotic and devitalized tissues [62,63]. The high amounts of carbohydrates including fructose, glucose, maltose, sucrose and other types are responsible for the high osmolarity of honey [9].

Although osmolarity of honey is an important anti-microbial factor, the anti-microbial activity of honey is not totally lost when honey is diluted [14]. For instance, it has been shown that honey

can be effective against *Staphylococcus aureus* when it is diluted by the wound exudates [52,64,65]. As a meaningful reason, when honey is diluted, it generates hydrogen peroxide which can inhibit bacterial growth [15]. In a study performed on patients with open or infected wounds, honey was more effective than sugar in reducing bacterial contamination and promoting wound healing.

2.1.1.3. Acidity

The range of acidic pH of honey is between 3.2 and 4.5 and this low pH is due to formation of gluconic acid [38,58]. During the dilution of honey, glucose oxidase catalyses glucose to gluconic acid and hydrogen peroxide where the gluconic acid results in acidity [5,66]. Honey provides an acidic environment which is unfavorable for bacterial growth and inhibits the activity of many micro-organisms [9,15,39,38,58,59,66,67].

In most instances, the wound pathogenic bacteria prefer neutral or slightly alkaline environments for growth [66]. On the other hand, the optimum pH for protease activity is about 7.3 [5]. Increased protease activity can destroy growth factors, newly regenerated collagenic matrix and fibronectin in wounds that are necessary for fibroblast activity and reepithelization leading to extra inflammatory reactions [15]. The acidity of honey has been suggested to reduce protease activity, increase fibroblast activity and oxygen release from hemoglobin in capillaries, decrease in wound size and ultimately promote wound healing [5,15,54,56,62,67,68-70]. Acidification also assists in the antibacterial action of macrophages and prevents ammonia produced by the bacterial metabolism to harm body tissues [71,72].

2.1.1.4. Non-peroxide activity and antioxidants

It has been found that antibacterial activity remains in honeys that are treated with catalase. One reason is the high level of non-peroxide antibacterial activity in honey. According to floral sources, harvest season and geographical location, honey may contain various components such as flavonoids and aromatic acids [57,70,73,74]. Several components such as methyl syringate, defensin-1 and MGO contribute to non-peroxide activity of honey [1,39,56]. Methylglyoxal is derived from the non-enzymatic conversion of dihydroxyacetone. This reaction occurs at high levels in nectar and its content increases with storage of honey at 37 ^{0}C [1,4,5]. MGO as an antimicrobial component is able to interact with the nucleophilic centers of macromolecules such as DNA. In gram-positive organisms, MGO downregulates autolysin enzyme that involves in cleavage of bacterial cell wall components and cell division. In gram-negative bacteria, MGO regulates gene expressions involved in stability of the cell wall [75].

It has been accepted that defensins have improving roles in antimicrobial activity of honeys. The defensins represent a family of cycteine-reach antimicrobial peptides that exist in two forms, defensin 1 and defensin 2 [76]. It is believed that defensin 2 acts as an inducible antibacterial peptide in bees and contributes to the antimicrobial activity of honeys [60]. Defensin 1 has 3 isoforms including defensin from hemolymph and 2 isoforms present in royal jelly that are called royalysin. These isoforms are encoded by one gene, but are different by one or two amino acid/s [76]. Bee defensin-1 has been identified in honey bee hemolymph, head and thoracic glands, royal jelly, the major food of queen bee larvae and also in honey [48,77].

Bee defensin-1 has potent activity against the mycelia fungi and yeast [78], protozoa [79], mites, viruses [80], gram-positive bacteria and some species of gram-negative bacteria [3,48,81]. However, defensin-1 may not have a great effectiveness on anti-biotic resistant bacteria. It has been shown that even at eight-fold higher concentrations; bee defensin-1 only slightly reduced

the survival of methicillin-resistant *Staphylococcus aureus* [41]. Interestingly, it has been shown that Lactobacillus non-pathogenic bacteria by increasing the expression level of abecin and defensins enhances the immunity of honeybee [82]. In fact defensins as a cycteine-reach antimicrobial peptide disrupt integrity and permeability of the cytoplasma membrane of pathogenic organisms [83].

2.1.1.5. Increased lymphocytic and phagocytic activity

The immune system is typically stimulated to immunize body against various infections and the use of honey can reflect more than just antibacterial properties. Honey is able to stimulate B-lymphocytes and T-lymphocytes, and activate neutrophils' phagocytosis in cell culture [15,58, 84,85]. Honey also stimulates monocytes (MM6 cells) to secrete cytokines, tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1) and IL-6, which activate the immune response to infection [39,68,84,86,87]. Secretion of TNF-α, a cytokine that induces the wound healing process, can be induced by glycosylated proteins. Furthermore, by reducing the release of reactive intermediates by activated macrophages during wound healing, honey is capable of degrading collagen IV through stimulation of the Matrix metalloproteinases 9 (MMP-9) during the re-epithelialization process of wound repair [88,89]. *In vitro* studies have shown that the toxicity of honey on keratinocytes and fibroblasts is extremely low [2 ,89]. It is believed that such immunomodulation effect further promotes the antimicrobial activity of honey [2].

2.1.1.6. Nitric Oxide

Nitric oxide (NO) is a gaseous free radical that has a wide range of activities such as regulation of angiogenesis, accelerating collagen synthesis and re-epithelialization in wound healing, antibacterial and antiviral effects, immunological and inflammatory responses, and initiating

ischemic cardiovascular and malignant diseases [90,91]. L-arginine, as a substrate for NO synthesis is metabolized in wound via arginase which exists in high concentrations in wound fluid. There are three isoforms of NO, the endothelial and neuronal forms are two constitutive isoforms and the last one is an inducible isoform (iNOS). The constitutive isoforms permanently generate low concentration of NO, while the iNOS form is induced by a variety of cytokines, growth factors and inflammatory stimuli [90,92]. All cells involved in wound healing, such as platelets, inflammatory cells, fibroblasts, and epithelial cells are able to produce NO [93]. Han et al. investigated the effects of a novel NO-releasing nanoparticle (NO-np) technology on wound healing in mice. They showed that NO-np can significantly promote angiogenesis and increase fibroblast migration and collagen deposition in wounded area [94]. Incorporation of NO into polymers can hasten wound healing processes and upregulate NO-inducible genes. Polymer-based NO therapies facilitate delivery to the target tissue and thereby cause reduce risk of toxic by-products leaching [95].

Heating and prolonged storage can decrease NO metabolites and thus lead to a reduction in antimicrobial activity of honey [57,95]. Al-Waili tested seven kinds of honey, different in their origin (three from Yemen, two from the United Arab Emirates, one from Germany, and one from India), color, and duration of storage, for the presence of nitrite/nitrate, the stable nitric oxide metabolites. All the honey samples had various concentrations of NO metabolites but the darker or fresh honeys contained more NO metabolites than the light or old honey. It has also been indicated that the darker old honey had more resistance to heating and ultraviolet exposure. They also evaluated the effects of intravenous infusion of honey on urinary and plasma NO end products in healthy sheep. Plasma NO metabolites increased during 1, 2, and 3 hours after infusion by 3%, 3.6%, and 17%, respectively and no side effects reported with the intravenous

administration of honey [96]. Honey increases NO end products in various biological fluids, such as urine, saliva, and plasma. Furthermore, NO production by honey could explain partly the effects of honey on immunity, bacterial infections, and wound healing [1,87,91].

2.1.1.7. Prostaglandins

Prostaglandins are mediators of inflammation and pain which are considered as immune-suppressives that can decrease B- and T-lymphocyte functions [57,91,97]. Honey can reduce plasma prostaglandin concentrations and its inhibitory effect increases with time [1,57,98]. Honey induced reduction in the concentration of prostaglandins may decrease edema and inflammation and activate lymphocytes to produce antibody against wound pathogens [91]. Honey also inhibits the cyclooxygenase-2 (COX-2) expression and the phenolic compounds of honey have a major role in inhibition of prostaglandin E2 (PGE2) in inflamed tissues. Ellagic acid, as a major phenolic compound in honey, prevents PGE2 release from monocytes and other phenolic compounds such as quercetin, chrysin and luteolin have inhibitory effects on interleukin 1β and COX-2 expression, PGE2 synthesis and nuclear factor-kappa beta (NF-κB) stimulation [91,99]. It has been shown that ingestion of diluted natural honey can reduce prostaglandin concentrations including PGE2, prostaglandin F2a (PGF2α) and thromboxane B2 in plasma of normal individuals [98,100]. Treatment of wounds with honey has been shown to less painful than sugar during dressing changes and motion [101].

2.1.1.8. Antibody production

Honey increases antibody production during primary and secondary immune responses against thymus-dependent and thymus-independent antigens [58]. Honey has an impact on antibody production by different mechanisms. However, the exact mechanism has not been well defined

as yet [57,102]. Honey is able to stimulate lymphocytes to produce cytokines, TNF-α, IL-1, and IL-16 [102]. Honey also might increase humoral immunity by increasing NO. On the other hand, by reducing the plasma prostaglandin concentration, honey results in antibody production [91, 98,102].

2.2. Debridement action

Debridement is a basic necessity to induce the functional process of tissue repair [7]. The standard procedure for the debridement of wounds is to surgically remove any dead tissue which is a painful procedure that should usually be performed under local analgesia. Likewise such method may cause growth of infecting bacteria and produces toxins which can destroy the surrounding tissues [8,58]. Application of honey as wound dressing provides a moist environment that induces rapid debridement of wounds [60,103,104]. The high osmotic pressure in honey and activation of proteases by the hydrogen peroxide found in honey may be two reasons for rapid debridement activity of honey. It also contributes to the painless lifting off of the slough and necrotic tissues [9]. In general, proteases are inactive in wound tissue and can be activated by oxidation. Proteases are also able to change the conformation of MMPs and make them active [13]. It may be thought that high protease activity impairs wound healing but such effect has never been proved. In fact potent anti-inflammatory action of honey can prevent excessive proteolytic activity [68].

An *In vitro* investigation showed that honey increases the activity of the enzyme plasmin in the culture medium. Plasmin digests the fibrin attached to the slough in wound surface, but does not digest the collagen matrix which is needed for tissue repair. Honey is able to inhibit production of the plasminogen activator inhibitor (PAI) by the macrophages. On the other hand,

inflammation increases production of PAI, thus reduction in production of PAI by honey is a good reason for its anti-inflammatory activity [84].

2.3. Anti-inflammatory activity

Inflammation is the biological response of body with the aiming to remove the harmful stimuli and initiate the healing process. In addition, inflammation is the major reason of pain and edema in wounds [58]. High concentration of the free radicals during the inflammatory phase of wound healing can extremely damage and break down lipids, proteins and nucleic acids that are essential components for the functional activities of all cells. Honey is able to reduce the damage caused by the free radicals and thus prevents further tissue necrosis [105,106]. The reactive oxygen species that are formed in the inflammatory phase, stimulate the activity of the fibroblasts. Fibroblasts produce the collagen fibers of scar tissue and prolongation of the inflammatory phase can lead to hyper-granulation and fibrosis. Therefore, reduction in the inflammatory phase by honey minimizes/prevents hypertrophic scarring [15,68,107,108].

In vitro studies have shown that honey stimulates monocytes to release TNF-α and cytokines such as IL-6 and IL-1β that may induce collagen synthesis by fibroblasts [55,56,68]. Some other studies have investigated the impact of phenolic components such as ellagic and gallic acids as anti-inflammatory agents [97,100,107]. However, there are several mechanisms including inhibition of the classical complement pathway, inhibition of ROS formation, inhibition of leukocyte infiltration, inhibition of MMP-9 and inhibition of COX-2 and inducible NO synthase expression which can describe the anti-inflammatory properties of honey [15,107]. It has been shown that honey inhibits the inflammatory process by inhibiting NF-κB pathway. Honey is able to translate NF-κB to the nucleus and decreases inflammatory mediators such as COX-2 and TNF-α [100,109]. To investigate the anti-inflammatory effect of honey in association with NF-

κB signaling pathway, Hissein et al. [109] evaluated the genes and proteins that are involved in this pathway. During inflammation, NF-κB actives and regulates transcription of IL-1β, IL-6, iNOS, COX-2 and TNF-α. To decrease the production of pro-inflammatory mediators, the major focus has been on inhibiting NF-κB activation and its translocation into the nucleus. The finding of this study showed that Gelam honey can block the incoming signal pathway which activates the IκB kinase (IKK) complex, and degrade IκB proteins and prevent the translocation of NF-κB subunits (p65 and p50) into nucleus and finally inhibit the iNOS, COX-2, TNF-α and IL-6 expressions as well as PGE2 and NO production [109]. It has been demonstrated that honey can act as an immune-modulator with both proinflammatory and anti-inflammatory properties. In low concentration of the inflammatory/stimulatory mediators, honey stimulates production of the inflammatory cytokines and MMP-9 from keratinocytes. On the other hand, when wound is infected and inflammation is in progress, honey inhibits production of the inflammatory cytokines and MMP-9 [107].

2.4. Boosting of the immune system

Peptides and proteins of honey can stimulate immune system by physiological responses in target cells via their specific receptors. For example the glycosylated proteins that induce TNF-α secretion from macrophages and also the 55 kDa glycoprotein major royal jelly protein 1 (MRJP1) have been suggested to be responsible for immunomodulatory effects and wound repair promoter mechanisms of honey. Another immune-stimulatory protein is type II arabinogalactan protein which is able to stimulate monocytic cells to release TNF-α [88,107].

The presence of NO in honey enables it to increase the humoral immunity. Honey also by decreasing the concentration of prostaglandins induces antibody production [1]. In addition, honey contains prebiotic oligosaccharides that enhance immune responses [1,110]. Recently,

Tonks et al. [111] reported that a 5.8 kDa component of honey is responsible for stimulating immune function *in vitro*. More studies are required to determine the nature of this compound.

2.5. Anti-oxidant activity

The antioxidant activity of natural honey is due to the presence of a variety of compounds such as flavonoids (apigenin, pinocembrin, kaempferol, quercetin, galangin, chrysin and hesperetin), phenolic acids (ellagic, caffeic, p-coumaric and ferulic acids), ascorbic acid, tocopherols, catalase, superoxide dismutase, reduced glutathione, maillard reaction products, peptides, amino acids and selenium [58,70,112-115]. The antioxidant effect depends on the relative positions of OH groups in the aromatic ring of phenolic acids. Moreover, the action of phenolic compounds can be related to their capacity to reduce and chelate ferric ion which catalyze lipid peroxidation [116]. Phenolics are the most important antioxidants in honey and among them; gallic acid is the most potent antioxidant [74]. There is a strong correlation between anti-oxidant activity and the content of total phenolics. Darker honeys have higher total phenolic- and antioxidant contents than lighter honeys [58,73,100,115]. In addition, the botanical origin of honey is an important factor in its antioxidant activity [107,117]. Proteins and sugars in the honey may form high molecular weight melanoidins that are the main components responsible for radical scavenging capacity [74,115].

It has been shown that ROSs mediate TNF-α-induced cytotoxicity and the phenolic compounds such as caffeic acid are able to effectively inhibit TNF-α-induced cytotoxicity *in vitro* [107]. In chronic wounds, neutrophils and macrophages release high levels of ROSs against invading bacteria. Prolonged exposure of a tissue to ROS causes cell damage and may retard wound healing. Therefore, application of antioxidants is a way to remove ROSs and honey contains several important antioxidant substances that can be used safely in wound healing [107]. In

addition, flavonoids of honey are also able to act as scavengers of ROS, peroxyl, alkyl peroxide, hydroxyl and superoxide radicals. These molecules protect tissue against reactive nitrogen species (RNS) such as NO and peroxynitrite [116]. Collectively, honey contains both aqueous and lipophilic antioxidants and thus can act at different cellular levels as an ideal natural antioxidant [15]. This activity reduces oxidative reactions by inhibiting the formation of free radicals such as iron and copper which are catalyzed by metal ions. This process leads to decrease in the oxidative stress and the inflammatory process [110,111].

2.6. Stimulation of wound regeneration and repair

Regeneration of a new tissue is an important step in wound healing process. Many studies have described the effectiveness of honey in the healing of both acute (burns, lacerations) and chronic wounds (venous leg ulcers, pressure ulcers) [4,118-123]. Honey by reduction of edema, subsiding inflammation and pain, facilitating debridement and deodorizing of the wounds, synthesis of collagen, development of new blood vessels, stimulating growth of fibroblasts and epithelial cells, promoting formation of granulation tissue, and preventing scar tissue and keloid formation can improve wound healing and minimizes the need for skin grafting so that no scab is developed and no excessive scarring and hypertrophication is formed [54,57,68,117].

Wound healing is arranged in three overlapping phases including inflammation, proliferation and remodeling [88,121]. In the inflammatory phase, honey stimulates monocytes to release inflammatory cytokines including TNF-α IL-6, IL-1β and NO that may induce collagen synthesis by fibroblasts [97]. These cytokines play major roles in the initiation and amplification of inflammatory processes [99]. The phenolic components contained in honey are frequently related to anti-inflammatory activity thus modulate the severity of inflammation [97]. Taken together, honey has modulatory role on inflammatory phase of wound healing so that it initiates an active

but controlled inflammation but does not let the inflammation to develop in a chronic or exaggerated state. Honey is able to remove debris and bacteria from the wound by stimulation of neutrophils, macrophages and phagocytosis during the inflammatory phase [97]. It has been shown that absence of macrophages leads to poor debridement of the wound site and delayed repair. In contrast, depletion of neutrophils leads to enhanced wound closure [111].

Angiogenesis, collagen deposition, granulation tissue formation, epithelialization, and wound contraction occur in the proliferative phase. Stimulation of angiogenesis by honey to supply the required oxygen in wound is an important feature in the healing process [55,68]. The acidic nature of honey releases oxygen from the hemoglobin, a mechanism that stimulates granulation tissue formation and wound healing.

On the other hand, honey accelerates wound contraction by stimulating fibroblasts, myofibroblasts and collagen deposition. It also promotes re-epithelialization and holds the wound edges together due to its high osmotic pressure [69]. Hydrogen peroxide contained at low levels in honey can stimulate the development of new capillaries [68,69], and growth and proliferation of fibroblasts and epithelial cells in wound tissue [55,124]. Al-Jadi et al. investigated the effect of a selected Malaysian honey and its major components on proliferation of cultured fibroblasts. They showed that hydrogen peroxide of honey can stimulate proliferation in a time- and dose-dependent manner. On the other hand, the presence of phenolics with antioxidant properties protected cells against the toxic effect of hydrogen peroxide. They also showed that honey and its major components are able to exert stimulatory effects on cultured fibroblasts [122].

Re-epithelialization is an important step in cutaneous wound healing which causes migration and proliferation of keratinocytes to skin surface. The enriched environment of honey provides

the required glucose as an energy package for the epithelial cells so that it facilitate their migration across the wound surface [68]. Honey also contains different trace elements (such as Zn, Fe, Cu, Co, Mn, and Mg) which are able to promote keratinocyte proliferation by modulating integrin expression during re-epithelialization [85]. MMPs and plasmin produced by immature keratinocytes separate keratinocytes from the basement membrane and also facilitate their migration [2,88]. In the remodeling phase, collagen is remodeled and realigned along tension lines and the cells that are no longer needed are removed by apoptosis (Fig. 2). Subrahmanyam et al. [123] have reported that honey can decrease scarring and contractures on burn patients and enhances remodeling of the cutaneous wounds.

3. Clinical observations on the wound healing properties of honey

To systematically evaluate the role of honey on clinical patients, only the clinical studies published in PubMed were considered. From the PubMed indexed clinical studies, only those that their patients had a cutaneous ulcer/wound and treated with honey, were included in the present review. In the present study, the clinical trials have been combined and their findings were provided as meta-analysis where appropriate and other clinical studies have been summarized, narratively. Based on the above approach, twenty-five studies were identified and included (Table 2) [72,87,101,125-146]. Fourteen of the studies involved chronic wounds (diabetic foot ulcers, venous ulcers and chronic wounds), nine studies evaluated acute wounds (burn and surgical wounds) and two studies investigated mixed chronic and acute wounds.

3.1. Acute wounds

Of the 25 studies, 9 of them (36%) recruited participants with acute wounds [72,125-132]. Two trials allocated participants with different acute wounds including one in lacerations or shallow abrasions, seven in burns and one in split-thickness skin grafts.

3.1.1. Burn wounds

Treatment of burn wounds is a significant problem in clinical practice [86]. Among clinical studies, seven (72,125-130) articles were conducted on burn patients. All controls were active comparisons and different types of agents were used in the clinical studies including silver sulphadiazine [72,127], potato peelings [128], amniotic membrane [129], tangential excision [126] and polyurethane film [130]. Of the seven studies, six were positive and showed that the use of honey significantly decreases times of healing and infection. One study [126] was negative and showed that tangential excision was significantly better than honey in the patients. Two studies reported a significant reduced mean healing time of 10.8 [130] and 13.47 [72] days with honey compared with 15.3 and 15.62 days with their treatments respectively (*P<0·0001*). Treatment with honey clearly improved the quality of wound healing at seven and fourteen days so that at fourteen days, 74.11% (229/302) of patients were healed with honey, and 57.89% (154/266) healed with other treatments. Based on the result of clinical studies, honey seems to heal burns more quickly than conventional treatments (Fig. 3, 4).

3.1.2. Other acute wounds

The Ingle et al. [131] trial recruited people with lacerations or shallow abrasions and treated control participants with a hydrogel. There were no significant differences in the mean healing time and wound size between groups treated with honey and IntraSite Gel. They believed that because there was no real difference between the groups, the natural form of honey could be an optimum cost-effective therapeutic option for management of the acute wounds [131]. In

contrast, Misirlioglu et al. [132] showed that in the treatment of split-thickness skin graft donor sites, honey-impregnated gauzes are better than paraffin gauzes and saline-soaked gauzes because of decreased epithelization time and a low sense of pain while there is no significant difference between honey-impregnated gauzes and hydrocolloid dressings with regard to epithelization time and sense of pain [132].

3.2. Chronic wounds

Of 25 included clinical trials, 14 (56%) studies recruited people with different chronic wounds including four in people with diabetic foot ulcers [87,133-135], five in venous leg ulcers [136-139], a single trial in pressure injuries [140] and another single trial in postoperative wound infections [141]. Three trials also recruited participants with different chronic wounds [142-144].

3.2.1. Diabetic foot ulcers

Four trials recruited people with diabetes and foot ulcers. Two [87,133] trials used randomized controlled trial strategy and compared the effects of honey with either povidone-iodine [133] or saline soaked gauze [87]. Although decreased mean healing time in both trials was observed, there was no significant difference in wound healing outcome between honey and the controls ($P>0.005$). It should be noted that slow healing of diabetic foot ulcer is due to poly-microbial infection, ischemia, neuropathy and immune-suppression. Based on these trials, honey leads to rapid disinfection of the ulcers and is able to reduce edema and foul smell from the ulcers.

In an article that was carried out by Moghazy et al. [134] thirty infected diabetic foot wounds were randomly selected. Based on their findings, 43.3% of the ulcers healed completely. Bacterial load significantly decreased in all the ulcers after the first week of honey dressing. In addition, healthy granulation tissue was regenerated and the wound size significantly decreased. This study concluded that although honey is ideal as dressing in cases of diabetic foot wounds

and after revascularization operation, it is not effective in cases of severe vascular compromise, exposed bone (without periosteum) or established osteomyelitis [134]. In another trial, Surahio et al. [135] evaluated the role of honey in 172 patients showing honey is able to significantly reduce rate of amputation and improve wound healing. Prolonged use of systemic antibiotics can develop drug resistant organisms in diabetic foot ulcers and honey can be considered as antibacterial agent which is suitable for the treatment of diabetic foot ulcers [135].

3.2.2. Venous leg ulcers

Among conducted studies, three trials with randomized controlled strategy used hydrogel [136], usual care [139] and phenytoin [140] as control groups. Compared with hydrogel treated group, honey exhibited an increased incidence of healing, more efficacious desloughing and lower rates of infection. Oluwatosin et al. [140], compared the effect of honey and phenytoin in the treatment of chronic leg ulcers. There was no significant difference in mean percent reduction in size between the honey treated-, phenytoin treated- and mixture treated groups. There was also no difference in pain score at the end of the four week treatment between the groups. These data suggest that phenytoin may be better than honey as a topical agent in the treatment of chronic ulcers [140]. In the Jull et al. [139] trial study, 55.6% of the participants in the honey-treated group and 49.7% in the usual care group healed. There was no significant difference in the healing percentage between the two groups ($p>0.05$) [139]. In other clinical trial studies, Schumacher et al. [137] and Dunford et al. [138] assessed the use of honey on treatment of patients with venous leg ulcers [137,138]. The Schumacher trial reported that the use of medical honey in patients who had received split-thickness skin grafts for chronic venous leg ulcers appeared to give a similar healing time to that seen without the application of honey [137]. Dunford and co-workers reported that the total average pain scores decreased significantly from

1.6 ±1.22 to 1.08 ±1.54 ($p<0.02$; paired Student's t-test) between baseline and the subjects' individual end-points and pain-free patients had the highest healing rates [138].

3.2.3. Other chronic wounds

One clinical trial study compared the effect of honey dressing versus ethoxy-diamino-acridine + nitrofurazone dressing in patients with pressure ulcers (PUs) [141]. In the honey treated group, there was a statistically significant mean decrease in ulcer size at 5 weeks when compared to the patients managed with ethoxy-diamino-acridine + nitrofurazone dressings (56% reduction *vs*. 13% reduction, $p<0.001$). In this study, 5 PUs in the honey group had healed at 5 weeks compared with no PU in the control group (20% *vs*. 0%; $p<0.05$). This study suggests that honey can significantly increase the healing rate when compared to the ethoxy-diamino-acridine + nitrofurazone dressing.

One clinical trial [142] analyzed the possible therapeutic effect of honey in the treatment of severe acute postoperative wound infections. In this study 22/26 (84.4%) participants in the honey group showed complete wound healing compared with 12/24 (50%) in the control group. This data suggests that honey can be effective in healing processes of the infected wounds.

Three trials recruited participants with different chronic wounds [143-145]. In the Robson et al. trial [143], the participants received either manuka honey or conventional dressing. 70% of the patients had venous leg ulcers. The median time to healing in the honey group was 100 days compared with 140 days in the control group and the healing rate at 12 weeks was 46.2% in the honey group compared with 34% in the conventional group. Based on this study, although the hazard ratio increased, the difference was not statistically significant ($p>0.05$) [143]. In the second clinical trial study, Gethin et al [144] suggested that the use of manuka honey dressings is associated with a statistically significant decrease in wound pH and a reduction in wound size so

that a reduction in 0.1 pH unit is associated with an 8.1% reduction in wound size [144]. Finally, Biglari et al. [145] demonstrated that honey has antibacterial activity and treatment with honey reduces pain and wound size and eliminates wound odor in the chronic wounds. They showed a relationship between pain and slough/necrosis at the time of recruitment and during wound healing [145].

Overall, since the chronic non-healing wounds have an elevated alkaline environment, the acidification of ulcer environment can propose as mechanism by which honey induce healing. The ability of the honey to maintain the moisture of the wounds is other possible explanation for the better healing outcome in chronic wounds (Fig 5).

3.3. Mixed acute and chronic wounds

Two trials evaluated the effects of honey in a range of different acute and chronic wounds [101,146]. In a quasi-randomized study, Mphande et al. [101] compared the role of honey and sugar in 40 patients with ulcers, chronic osteomyelitis, post-surgical and traumatic wounds. The median healing rate was 1.43 days/cm^2 for the honey group and 1.62 days/cm^2 for the sugar group. Also, during dressing changes, there was no pain in 86% of the patients treated with honey, compared with 72% of the patients treated with sugar [101]. In another study, Ahmed et al. [146] evaluated the role of honey in 60 patients with chronic, complicated surgical or acute traumatic wounds. Complete healing of wounds was obtained in 96.6% of the patients treated with honey [146]. Overall, they suggested that honey is an effective, safe, and economic wound-dressing medium for the treatment of acute or chronic wounds. There were no signs of allergy, not even after extended use of this dressing material.

4. Conclusion

In the present review, we highlighted the key information regarding therapeutic properties of honey and its mechanism of action. Honey is the most ancient wound dressing biomaterial and the effectiveness of honey in the management of wounds has been confirmed by many studies. There is no allergic reaction and no significant side effects due to clinical application of honey. Honey has all of the beneficial criteria to be considered as an ideal dressing material applicable for various wounds. It is believed that honey could be used as a suitable alternative option in most of the infected wounds due to its anti-bacterial and wound healing activity which are due to many factors including acidity, hydrogen peroxide content, osmolarity and phytochemical components. The antibacterial, anti-inflammatory and antioxidant properties, as well as debridement action, boosting the immune system and stimulation of tissue growth, make it a logical and accepted natural agent for wound dressing. Honey is able to facilitate the wound healing processes by stimulating the epithelialization, improving angiogenesis, facilitating wound contraction, and increasing collagen synthesis and wound debridement. It should be remembered that because bees have different nutritional behavior and collect the nourishments from different and various plants, the produced honeys have different compositions. Thus different types of honey have different medicinal value leading to different effects on wound healing. In a meta-analyses we performed in this review, most of the clinical trials suggested the use of honey for management of various wound models including lacerations, shallow abrasions, burns, large defects, diabetic foot ulcers, venous leg ulcers, pressure ulcers and postoperative wound infections. Based on the results of the present study, honey is suggested as a safe, cost-effective and beneficial dressing biomaterial for wound management however the clinicians should aware about the composition and biological properties of the selected honey before medical application. Future studies are appreciated if compare the composition, characteristics

and biological properties of different kind of honeys in order to illustrate a guide line for medical application.

References

[1] Al-Waili NS, Salom K, Butler G, Al Ghamdi AA. Honey and microbial infections: a review supporting the use of honey for microbial control. J Med Food 2011;14(10):1079–96.

[2] Lee DS, Sinno S, Khachemoune A. Honey and wound healing: an overview. Am J Clin Dermatol 2011;12(3):181–90.

[3] Kwakman PH, Zaat SA. Antibacterial components of honey. IUBMB Life 2012;64(1):48–55.

[4] Oryan A, Zaker SR. Effects of topical application of honey on cutaneous wound healing in rabbits. Zentralbl Veterinarmed A 1998;45(3):181–8.

[5] Bittmann S, Luchter E, Thiel M, Kameda G, Hanano R, Längler A. Does honey have a role in paediatric wound management? Br J Nurs 2010;19(15):S19–20,S22,S24.

[6] Moore OA, Smith LA, Campbell F, Seers K, McQuay HJ, Moore RA. Systematic review of the use of honey as a wound dressing. BMC Complement Altern Med 2001;1:2.

[7] Strohal R. The EWMA document: debridement. J Wound Care 2013;22(1):5.

[8] Rüttermann M, Maier-Hasselmann A, Nink-Grebe B, Burckhardt M. Local treatment of chronic wounds: in patients with peripheral vascular disease, chronic venous insufficiency, and diabetes. Dtsch Arztebl Int 2013;110(3):25–31.

[9] Manyi-Loh CE, Clarke AM, Ndip RN. An overview of honey: Therapeutic properties and contribution in nutrition and human health. Afr J Microbiol Res 2011;5(8):844–52.

[10] Wasiak J, Cleland H, Campbell F. Dressings for superficial and partial thickness burns. Cochrane Database Syst Rev 2008;(4):CD002106.

[11] Oryan A, Khalafi-Nezhad A, Toloo N, Soltani Rad MN. Effects of 4-chloro-2,6-bis-(2-hydroxyl-benzyl)-phenol on healing of skin wounds and growth of bacteria. J Vet Med A Physiol Pathol Clin Med 2007;54(10):585–91.

[12] Oryan A, Tabatabaeinaeini A, Nikahval B, Gorgian E. Effects of aqueous extract of *Aloe vera* on experimental cutaneous wound healing in rat. Vet Arhiv 2010;80(4):509–22.

[13] Tabandeh MR, Oryan A, Mohhammad-Alipour A, Tabatabaei-Naieni A. Silibinin regulates matrix metalloproteinase 3 (stromelysine1) gene expression, hexoseamines and collagen production during rat skin wound healing. Phytother Res 2013;27(8):1149–53.

[14] Song JJ, Salcido R. Use of honey in wound care: an update. Adv Skin Wound Care 2011;24(1):40–4.

[15] Yaghoobi R, Kazerouni A, Kazerouni O. Evidence for clinical use of honey in wound healing as an anti-bacterial, anti-inflammatory anti-oxidant and anti-viral agent: a review. Jundishapur J Nat Pharm Prod 2013;8(3):100–4.

[16] Liu JR, Ye YL, Lin TY, Wang YW, Peng CC. Effect of floral sources on the antioxidant, antimicrobial, and anti-inflammatory activities of honeys in Taiwan. Food Chem 2013;139(1–4):938–43.

[17] Brudzynski K, Abubaker K, Wang T. Powerful bacterial killing by buckwheat honeys is concentration-dependent, involves complete DNA degradation and requires hydrogen peroxide. Front Microbiol 2012;4(3):242.

[18] Fair RJ, Tor Y. Antibiotics and bacterial resistance in the 21st century. Perspect Medicin Chem 2014;28(6):25–64.

[19] Tirado DJ, Hudson NR, Maldonado CJ. Efficacy of medical grade honey against multidrug-resistant organisms of operational significance: Part I. J Trauma Acute Care Surg 2014;77(3 Suppl 2):S204–7.

[20] Cooper R, Jenkins R. Are there feasible prospects for manuka honey as an alternative to conventional antimicrobials? Expert Rev Anti Infect Ther 2012;10(6):623–5.

[21] Labban L, Honey as a promising treatment for diabetic foot ulcers (DFU). J Med Soc 2014;28(2):64–8.

[22] McLoone P, Warnock M, Fyfe L. Honey: A realistic antimicrobial for disorders of the skin. J Microbiol Immunol Infect 2015. pii: S1684-1182(15)00033-X.

[23] Blair SE, Cokcetin NN, Harry EJ, Carter DA. The unusual antibacterial activity of medical-grade Leptospermum honey: antibacterial spectrum, resistance and transcriptome analysis. Eur J Clin Microbiol Infect Dis 2009;28(10):1199–208.

[24] Camplin AL, Maddocks SE. Manuka honey treatment of biofilms of *Pseudomonas aeruginosa* results in the emergence of isolates with increased honey resistance. Ann Clin Microbiol Antimicrob 2014;12(13):19.

[25] Cooper RA, Jenkins L, Henriques AF, Duggan RS, Burton NF. Absence of bacterial resistance to medical-grade manuka honey. Eur J Clin Microbiol Infect Dis 2010;29(10):1237–41.

[26] Brudzynski K, Sjaarda C. Antibacterial compounds of Canadian honeys target bacterial cell wall inducing phenotype changes, growth inhibition and cell lysis that resemble action of β-lactam antibiotics. PLoS One 2014;9(9):e106967.

[27] Majtan J, Bohova J, Horniackova M, Klaudiny J, Majtan V. Anti-biofilm effects of honey against wound pathogens *Proteus mirabilis* and *Enterobacter cloacae*. Phytother Res 2014;28(1):69–75.

[28] Lu J, Turnbull L, Burke CM, Liu M, Carter DA, Schlothauer RC, et al. Manuka-type honeys can eradicate biofilms produced by *Staphylococcus aureus* strains with different biofilm-forming abilities. PeerJ 2014;2:e326.

[29] Ansari MJ, Al-Ghamdi A, Usmani S, Al-Waili NS, Sharma D, Nuru A, et al. Effect of jujube honey on *Candida albicans* growth and biofilm formation. Arch Med Res 2013;44(5):352–60.

[30] Merckoll P, Jonassen TØ, Vad ME, Jeansson SL, Melby KK. Bacteria, biofilm and honey: a study of the effects of honey on 'planktonic' and biofilm-embedded chronic wound bacteria. Scand J Infect Dis 2009;41(5):341–7.

[31] Atrott J, Henle T. Methylglyoxal in Manuka honey – correlation with antibacterial properties. Czech J Food Sci 2009;27:S163–5.

[32] Stephens JM, Schlothauer RC, Morris BD, Yang D, Fearnley L, Greenwood DR, et al. Phenolic compounds and methylglyoxal in some New Zealand manuka and kanuka honeys. Food Chem 2010;120(1):78–86.

[33] Majtan J. Methylglyoxal-a potential risk factor of manuka honey in healing of diabetic ulcers. Evid Based Complement Alternat Med 2011;2011:295494.

[34] Katiraee F, Mahmodi R, Mardani K, Babaei E. Antifungal activity of Iranian honeybees against *Candida, Aspergillus* species and *Trichophyton Rubrum*. J Food Process Preserv 2014;38(5):2078–82.

[35] Canonico B, Candiracci M, Citterio B, Curci R, Squarzoni S, Mazzoni A, et al. Honey flavonoids inhibit *Candida albicans* morphogenesis by affecting DNA behavior and mitochondrial function. Future Microbiol 2014;9(4):445–56.

[36] Zahoor M, Naz S, Sangeen M. Antibacterial, antifungal and antioxidant activities of honey collected from Timergara (Dir, Pakistan). Pak J Pharm Sci 2014;27(1):45–50.

[37] Brudzynski K, Abubaker K, St-Martin L, Castle A. Re-examining the role of hydrogen peroxide in bacteriostatic and bactericidal activities of honey. Front Microbiol 2011;2:213.

[38] Knight A. The therapeutic effects of honey. The Plymouth Student Scientist 2013;6(1):376–85.

[39] Mandal MD, Mandal S. Honey: its medicinal property and antibacterial activity. Asian Pac J Trop Biomed 2011;1(2):154–60.

[40] Bang LM, Buntting C, Molan P. The effect of dilution on the rate of hydrogen peroxide production in honey and its implications for wound healing. J Altern Complement Med 2003;9(2):267–73.

[41] Kwakman PH, de Boer L, Ruyter-Spira CP, Creemers-Molenaar T, Helsper JP, Vandenbroucke-Grauls CM, et al. Medical-grade honey enriched with antimicrobial peptides has enhanced activity against antibiotic-resistant pathogens. Eur J Clin Microbiol Infect Dis 2011;30(2):251–7.

[42] Banaeian-Borujeni S, Mobini GR, Pourgheysari B, Validi M. Comparison of the effect of honey and miconazole against *Candida albicans in vitro*. Adv Biomed Res 2013;30(2):57.

[43] White JW, Subers MH, Schepartz AI. The identification of inhibine, the antibacterial factor in honey, as hydrogen peroxide and its origin in a honey glucose–oxidase system. Biochim Biophys Acta 1963;73:57–70.

[44] Schepartz AI, Subers MH. The glucose-oxidase of honey: I. Purification and some general properties of the enzyme. Biochim Biophys Acta 1964;85:228–37.

[45] Brudzynski K. Effect of hydrogen peroxide on antibacterial activities of Canadian honeys. Can J Microbiol 2006;52(12):1228–37.

[46] Chen C, Campbell LT, Blair SE, Carter DA. The effect of standard heat and filtration processing procedures on antimicrobial activity and hydrogen peroxide levels in honey. Front Microbiol 2012;3:265.

[47] Majtan J, Bohova J, Prochazka E, Klaudiny J. Methylglyoxal may affect hydrogen peroxide accumulation in manuka honey through the inhibition of glucose oxidase. J Med Food 2014;17(2):290–3.

[48] Irish J, Blair S, Carter DA. The antibacterial activity of honey derived from Australian flora. PLoS One 2011;6(3):e18229.

[49] Kretavičius J, Kurtinaitienė B, Račys J, Čeksterytė V. Inactivation of glucose oxidase during heat-treatment de-crystallization of honey. Žemdirbystė (Agriculture) 2010;97(4):115–22.

[50] Majtan J, Klaudiny J, Bohova J, Kohutova L, Dzurova M, Sediva M, et al. Methylglyoxal-induced modifications of significant honeybee proteinous components in manuka honey: Possible therapeutic implications. Fitoterapia 2012;83(4):671–7.

[51] Molan PC. The antibacterial activity of honey. 2. Variation in the potency of the anibacterial activity. Bee World 1992;73(2):59–76.

[52] Kwakman PH, Te Velde AA, de Boer L, Vandenbroucke-Grauls CM, Zaat SA. Two major medicinal honeys have different mechanisms of bactericidal activity. PLoS One 2011;6(3):e17709.

[53] Bucekova M, Valachova I, Kohutova L, Prochazka E, Klaudiny J, Majtan J. Honeybee glucose oxidase--its expression in honeybee workers and comparative analyses of its content and H_2O_2-mediated antibacterial activity in natural honeys. Naturwissenschaften 2014;101(8):661–70.

[54] Molan PC. Potential of honey in the treatment of wounds and burns. Am J Clin Dermatol 2001;2(1):13–9.

[55] Rossiter K, Cooper AJ, Voegeli D, Lwaleed BA. Honey promotes angiogeneic activity in the rat aortic ring assay. J Wound Care 2010;19(10):440,442–6.

[56] Sell SA, Wolfe PS, Spence AJ, Rodriguez IA, McCool JM, Petrella RL, et al. A preliminary study on the potential of manuka honey and platelet-rich plasma in wound healing. Int J Biomater 2012;2012:313781.

[57] Al-Waili N, Salom K, Al-Ghamdi AA. Honey for wound healing, ulcers, and burns; data supporting its use in clinical practice. ScientificWorldJournal 2011;11:766–87.

[58] Singh MP, Chourasia HR, Agarwal M, Malhotra A, Sharma M, Sharma D, et al. Honey as complementary medicine: A review. IJPBS 2012;3(2):12–31.

[59] Aggad H, Guemour D. Honey Antibacterial Activity. Med Aromat Plants 2014;3:2.

[60] Maddocks SE, Jenkins RE. Honey: a sweet solution to the growing problem of antimicrobial resistance? Future Microbiol 2013;8(11):1419–29.

[61] George NM, Cutting KF. Antibacterial honey (Medihoney): *in vitro* activity against clinical isolates of MRSA, VRE, and other multi resistant gram-negative organisms including *Pseudomonas aeruginosa*. Wounds 2007;19(9):231–6.

[62] Stewart JA, McGrane OL, Wedmore IS. Wound care in the wilderness: is there evidence for honey? Wilderness Environ Med 2014;25(1):103–10.

[63] Alam F, Islam MA, Gan SH, Khalil MI. Honey: a potential therapeutic agent for managing diabetic wounds. Evid Based Complement Alternat Med 2014;2014:169130.

[64] Karayil S, Deshpande SD, Koppikar GV. Effect of honey on multidrug resistant organisms and its synergistic action with three common antibiotics. J Postgrad Med 1998;44(4):93–6.

[65] Cooper RA, Molan PC, Harding KG. Antibacterial activity of honey against strains of *Staphylococcus aureus* from infected wounds. J Roy Soc Med 1999;92(2):283–5.

[66] Ortiz-Vázquez E, Cuevas-Glory L, Zapata-Baas G, Martínez-Guevara J, Ramón-Sierra J. Which bee honey components contribute to its antimicrobial activity? A review. Afr J Microbiol Res 2013;7(51):5758–65.

[67] Schreml S, Szeimies RM, Karrer S, Heinlin J, Landthaler M, Babilas P. The impact of the pH value on skin integrity and cutaneous wound healing. J Eur Acad Dermatol Venereol 2010;24(4):373–8.

[68] Molan PC. Re-introducing honey in the management of wounds and ulcers - theory and practice. Ostomy Wound Manage 2002;48(11):28–40.

[69] Nakajima Y, Nakano Y, Fuwano S, Hayashi N, Hiratoko Y, Kinoshita A, et al. Effects of three types of Japanese honey on full-thickness wound in mice. Evid Based Complement Alternat Med 2013;2013:504537.

[70] Vandamme L, Heyneman A, Hoeksema H, Verbelen J, Monstrey S. Honey in modern wound care: a systematic review. Burns 2013;39(8):1514–25.

[71] Molan PC. Why honey is effective as a medicine 2. The scientific explanation of its effects. Bee World. 2001;82(1):22–40.

[72] Malik KI, Malik MA, Aslam A. Honey compared with silver sulphadiazine in the treatment of superficial partial-thickness burns. Int Wound J 2010;7(5):413–7.

[73] Moniruzzaman M, Sulaiman SA, Khalil MI, Gan SH. Evaluation of physicochemical and antioxidant properties of sourwood and other Malaysian honeys: a comparison with manuka honey. Chem Cent J 2013;7:138.

[74] Kus PM, Congiu F, Teper D, Sroka Z, Jerković I, Tuberoso CIG. Antioxidant activity, color characteristics, total phenol content and general HPLC fingerprints of six Polish unifloral honey types. LWT - Food Sci Technol 2014;55(1):124–30.

[75] Dart AJ, Bischofberger AS, Dart CM, Jeffcott LB. A review of research into second intention equine wound healing using manuka honey: Current recommendations and future applications. Equine Vet Educ 2015.doi: 10.1111/eve.12379.

[76] Il'iasov RA, Gaĭfullina LR, Saltykova ES, Poskriakov AV, Nikolenko AG. Defensins in the honeybee antinfectious protection. Zh Evol Biokhim Fiziol 2012;48(5):425–432.

[77] Kwakman PH, te Velde AA, de Boer L, Speijer D, Vandenbroucke-Grauls CM, Zaat SA. How honey kills bacteria. FASEB J 2010;24(7):2576–82.

[78] Aronstein KA, Murray KD, Saldivar E. Transcriptional Responses in Honeybee Larvae Infected with Chalkbrood Fungus. BMC Genom 2010;11:391.

[79] Antunez K, Martin-Hernandez R, Prieto L, Meana A, Zunino P, Higes M. Immune Suppression in the Honeybee (*Apis mellifera*) Following Infection by *Nosema ceranae* (Microsporidia). Environ Microbiol 2009;11(9):2284–90.

[80] Williams GR, Rogers RE, Kalkstein AL, Taylor BA, Shutler D, Ostiguy N. Deformed Wing Virus in Western Honeybees (*Apis mellifera*) from Atlantic Canada, the First Description of an Overtly-infected Emerging Queen. J Invertebr Pathol 2009;101(1):77–9.

[81] Bachanova K, Klaudiny J, Kopernicky J, Simuth J. Identification of honeybee peptide active against Paenibacillus larvae through bacterial growth-inhibition assay on polyacrylamide gel. Apidologie 2002;33(3):259–69.

[82] Arbia KA, Babbay B. Management strategies of honeybee diseases. J Entomol 2011;8:1–15.

[83] McDermott AM. Defensins and other antimicrobial peptides at the ocular surface. Ocul Surf 2004;2(4):229–47.

[84] Molan PC. The evidence and the rationale for the use of honey as a wound dressing. Wound Practice and Research 2011;19(4):204–20.

[85] Barui A, Mandal N, Majumder S, Das RK, Sengupta S, Banerjee P, et al. Assessment of molecular events during in vitro re-epithelialization under honey-alginate matrix ambience. Mater Sci Eng C Mater Biol Appl 2013;33(6):3418–25.

[86] Jastrzębska-Stojko Z, Stojko R, Rzepecka-Stojko A, Kabała-Dzik A, Stojko J. Biological activity of propolis-honey balm in the treatment of experimentally-evoked burn wounds. Molecules 2013;18(11):14397–413.

[87] Kamaratos AV, Tzirogiannis KN, Iraklianou SA, Panoutsopoulos GI, Kanellos IE, Melidonis AI. Manuka honey-impregnated dressings in the treatment of neuropathic diabetic foot ulcers. Int Wound J 2014;11(3):259–63.

[88] Majtan J, Kumar P, Majtan T, Walls AF, Klaudiny J. Effect of honey and its major royal jelly protein 1 on cytokine and MMP-9 mRNA transcripts in human keratinocytes. Exp Dermatol 2010;19(3):e73–9.

[89] Burlando B, Cornara L. Honey in dermatology and skin care: a review. J Cosmet Dermatol 2013;12(4):306–13.

[90] Witte MB, Barbul A. Role of nitric oxide in wound repair. Am J Surg 2002;183(4):406–12.

[91] Kassim M, Achoui M, Mansor M, Yusoff KM. The inhibitory effects of Gelam honey and its extracts on nitric oxide and prostaglandin E(2) in inflammatory tissues. Fitoterapia 2010;81(8):1196–201.

[92] Luo JD, Chen AF. Nitric oxide: a newly discovered function on wound healing. Acta Pharmacol Sin 2005;26(3):259–64.

[93] Lowe A, Bills J, Verma R, Lavery L, Davis K, Balkus KJ Jr. Electrospun nitric oxide releasing bandage with enhanced wound healing. Acta Biomater 2015;13:121–30.

[94] Han G, Nguyen LN, Macherla C, Chi Y, Friedman JM, Nosanchuk JD, et al. Nitric oxide-releasing nanoparticles accelerate wound healing by promoting fibroblast migration and collagen deposition. Am J Pathol 2012;180(4):1465–73.

[95] Brisbois EJ, Bayliss J, Wu J, Major TC, Xi C, Wang SC, et al. Optimized polymeric film-based nitric oxide delivery inhibits bacterial growth in a mouse burn wound model. Acta Biomater 2014;10(10):4136–42.

[96] Al-Waili NS. Identification of nitric oxide metabolites in various honeys: effects of intravenous honey on plasma and urinary nitric oxide metabolites concentrations. J Med Food 2003;6(4):359–64.

[97] Raynaud A, Ghezali L, Gloaguen V, Liagre B, Quero F, Petit JM. Honey-induced macrophage stimulation: AP-1 and NF-κB activation and cytokine production are unrelated to LPS content of honey. Int Immunopharmacol 2013;17(3):874–9.

[98] Al-Waili NS, Boni NS. Natural honey lowers plasma prostaglandin concentrations in normal individuals. J Med Food 2003;6(2):129–33.

[99] Hussein SZ, Mohd Yusoff K, Makpol S, Mohd Yusof YA. Gelam honey inhibits the production of proinflammatory, mediators NO, PGE(2), TNF-α, and IL-6 in carrageenan-induced acute paw edema in rats. Evid Based Complement Alternat Med 2012;2012:109636.

[100] Vallianou NG, Gounari P, Skourtis A, Panagos J, Kazazis C. Honey and its anti-inflammatory, anti-bacterial and anti-oxidant properties. General Med 2014;2(2):132.

[101] Mphande AN, Killowe C, Phalira S, Jones HW, Harrison WJ. Effects of honey and sugar dressings on wound healing. J Wound Care 2007;16(7):317–19.

[102] Al-Waili NS, Haq A. Effect of honey on antibody production against thymus-dependent and thymus-independent antigens in primary and secondary immune responses. J Med Food 2004;7(4):491–4.

[103] Sukur SM, Halim AS, Singh KK. Evaluations of bacterial contaminated full thickness burn wound healing in Sprague Dawley rats treated with Tualang honey. Indian J Plast Surg 2011;44(1):112–7.

[104] Sazegar G, Seyed Reza AH, Behravan E. The effects of supplemental zinc and honey on wound healing in rats. Iran J Basic Med Sci 2011;14(4):391–8.

[105] Alzahrani HA, Boukraa L, Bellik Y, Abdellah F, Bakhotmah BA, Kolayli S, et al. Evaluation of the antioxidant activity of three varieties of honey from different botanical and geographical origins. Glob J Health Sci 2012;4(6):191–6.

[106] Kishore RK, Halim AS, Syazana MS, Sirajudeen KN. Tualang honey has higher phenolic content and greater radical scavenging activity compared with other honey sources. Nutr Res 2011;31(4):322–5.

[107] Majtan J. Honey: an immunomodulator in wound healing. Wound Repair Regen 2014;22(2):187–92.

[108] Tasleem S, Naqvi SB, Khan SA, Hashimi K. Honey ointment: a natural remedy of skin wound infections. J Ayub Med Coll Abbottabad 2011;23(2):26–31.

[109] Hussein SZ, Mohd Yusoff K, Makpol S, Mohd Yusof YA. Gelam honey attenuates carrageenan-induced rat paw inflammation via NF-κB pathway. PLoS One 2013;8(8):e72365.

[110] Chepulis LM. The Effects of honey compared with sucrose and a sugar-free diet on neutrophil phagocytosis and lymphocyte numbers after long-term feeding in rats. J Complement Integr Med 2007;4(1):1–7.

[111] Tonks AJ, Dudley E, Porter NG, Parton J, Brazier J, Smith EL, et al. A 5.8-kDa component of manuka honey stimulates immune cells via TLR4. J Leukoc Biol 2007;82(5):1147–55.

[112] Jubri Z, Rahim NB, Aan GJ. Manuka honey protects middle-aged rats from oxidative damage. Clinics (Sao Paulo) 2013;68(11):1446–54.

[113] Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OJ. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chem 2005;91(3):571–7.

[114] Pourreza N. Phenolic Compounds as potential antioxidant. Jundishapur J Nat Pharm Prod 2013;8(4):149–50.

[115] Saric G, Markovic K, Vukicevic D, Vahcić N. Changes of antioxidant activity in honey after heat treatment. Czech J Food Sci 2013;31(6):601–6.

[116] Alvarez-Suarez JM, Giampieri F, Battino M. Honey as a source of dietary antioxidants: structures, bioavailability and evidence of protective effects against human chronic diseases. Curr Med Chem 2013;20(5):621–38.

[117] Al-Mamarya M, Al-Meerib A, Al-Haborib M. Antioxidant activities and total phenolics of different types of honey. Nutrition Research 2002;22(9):1041–7.

[118] Maleki H, Gharehaghaji AA, Dijkstra PJ. A novel honey-based nanofibrous scaffold for wound dressing application. J Appl Polym Sci 2012;127(5):4086–92.

[119] Shirvani MA, Nikpour M, Azadbakht M, Banihosseini SZ, Zanjani R. The effect of honey gel on cesarean incision pain: A triple blind clinical trial. Afr J Pharm Pharmacol 2013;7(1):19–24.

[120] Gulati S, Qureshi A, Srivastava A, Kataria K, Kumar P, Ji AB. A prospective randomized study to compare the effectiveness of honey dressing vs. povidone iodine dressing in chronic wound healing. Indian J Surg 2014;76(3):193–8.

[121] Tan MK, Hasan Adli DS, Tumiran MA, Abdulla MA, Yusoff KM. The efficacy of Gelam honey dressing towards excisional wound healing. Evid Based Complement Alternat Med 2012;2012:805932.

[122] Al-Jadi AM, Kanyan Enchang F, Mohd Yusoff K. The effect of Malaysian honey and its major components on the proliferation of cultured fibroblasts. Turk J Med Sci 014;44(5):733–40.

[123] Subrahmanyam M, Shahapure AG, Nagne NS. Bhagwat VR, Ganu JV. Effects of topical application of honey on burn wound healing. Ann Burns Fire Disasters 2001;14:143–5.

[124] Boekema BK, Pool L, Ulrich MM. The effect of a honey based gel and silver sulphadiazine on bacterial infections of *in vitro* burn wounds. Burns 2013;39(4):754–9.

[125] Nasir NA, Halim AS, Singh KK, Dorai AA, Haneef MN. Antibacterial properties of tualang honey and its effect in burn wound management: a comparative study. BMC Complement Altern Med 2010;10:31.

[126] Subrahmanyam M. Early tangential excision and skin grafting of moderate burns is superior to honey dressing: a prospective randomised trial. Burns 1999;25(8):729–31.

[127] Subrahmanyam M. A prospective randomised clinical and histological study of superficial burn wound healing with honey and silver sulfadiazine. Burns 1998;24(2):157–61.

[128] Subrahmanyam M. Honey dressing versus boiled potato peel in the treatment of burns: a prospective randomized study. Burns 1996;22(6):491–3.

[129] Subrahmanyam M. Honey-impregnated gauze versus amniotic membrane in the treatment of burns. Burns 1994;20(4):331–3.

[130] Subrahmanyam M. Honey impregnated gauze versus polyurethane film (OpSite) in the treatment of burns--a prospective randomised study. Br J Plast Surg 1993;46(4):322–3.

[131] Ingle R, Levin J, Polinder K. Wound healing with honey--a randomised controlled trial. S Afr Med J 2006;96(6):831–5.

[132] Misirlioglu A, Eroglu S, Karacaoglan N, Akan M, Akoz T, Yildirim S. Use of honey as an adjunct in the healing of split-thickness skin graft donor site. Dermatol Surg 2003;29(2):168–72.

[133] Shukrimi A, Sulaiman AR, Halim AY, Azril A. A comparative study between honey and povidone iodine as dressing solution for Wagner type II diabetic foot ulcers. Med J Malaysia 2008;63(1):44–6.

[134] Moghazy AM, Shams ME, Adly OA, Abbas AH, El-Badawy MA, Elsakka DM, et al. The clinical and cost effectiveness of bee honey dressing in the treatment of diabetic foot ulcers. Diabetes Res Clin Pract 2010;89(3):276–81.

[135] Surahio AR, Khan AA, Farooq M, Fatima I. Role of honey in wound dressing in diabetic foot ulcer. J Ayub Med Coll Abbottabad 2014;26(3):304–6.

[136] Gethin G, Cowman S. Bacteriological changes in sloughy venous leg ulcers treated with manuka honey or hydrogel: an RCT. J Wound Care 2008;17(6):241–4,246–7.

[137] Schumacher HH. Use of medical honey in patients with chronic venous leg ulcers after split-skin grafting. J Wound Care 2004;13(10):451–2.

[138] Dunford CE, Hanano R. Acceptability to patients of a honey dressing for non-healing venous leg ulcers. J Wound Care 2004;13(5):193–7.

[139] Jull A, Walker N, Parag V, Molan P, Rodgers A. Honey as adjuvant leg Ulcer therapy trial collaborators. Randomized clinical trial of honey-impregnated dressings for venous leg ulcers. Br J Surg 2008;95(2):175–82.

[140] Oluwatosin OM, Olabanji JK, Oluwatosin OA, Tijani LA, Onyechi HU. A comparison of topical honey and phenytoin in the treatment of chronic leg ulcers. Afr J Med Med Sci 2000;29(1):31–4.

[141] Yapucu Güneş U, Eşer I. Effectiveness of a honey dressing for healing pressure ulcers. J Wound Ostomy Continence Nurs 2007;34(2):184–90.

[142] Al-Waili NS, Saloom KY. Effects of topical honey on post-operative wound infections due to gram positive and gram negative bacteria following caesarean sections and hysterectomies. Eur J Med Res 1999;4(3):126–30.

[143] Robson V, Dodd S, Thomas S. Standardized antibacterial honey (Medihoney) with standard therapy in wound care: randomized clinical trial. J Adv Nurs 2009;65(3):565–75.

[144] Gethin GT, Cowman S, Conroy RM. The impact of Manuka honey dressings on the surface pH of chronic wounds. Int Wound J 2008;5(2):185–94.

[145] Biglari B, Moghaddam A, Santos K, Blaser G, Büchler A, Jansen G, et al. Multicentre prospective observational study on professional wound care using honey (Medihoney™). Int Wound J 2013;10(3):252–9.

[146] Ahmed AK, Hoekstra MJ, Hage JJ, Karim RB. Honey-medicated dressing: transformation of an ancient remedy into modern therapy. Ann Plast Surg 2003;50(2):143–8.

Table 1. Summary of the honey's therapeutic properties and effects of each property on wound healing

Properties	Effective factors	Actions	References
Antimicrobial activity	- Hydrogen peroxide - High osmolarity - Acidity - Non-peroxide activity - Antioxidants - Increased lymphocytic and phagocytic activity - Nitric oxide - Reduces prostaglandins	- Inhibits fungal growth and toxin production. - Inhibits viral infection - Affects on bacterial biofilms	1,5
Anti-inflammatory activity	- Leucocytes - Phenolic components	- Reduces edema and pain. - Minimizes hypertrophic scarring. - Stimulates granulation and epithelialization. - Improves oxygenation. - Prevents necrosis. - enhances collagen synthesis. - Inhibits leukocyte infiltration.	5,9,15,58,97,107
Anti-oxidant activity	- Flavonoids - Phenolic acids	- Radical scavenging - Inhibits TNF-α-induced cytotoxicity. - Decreases number of inflammatory cells - Stimulates B and T lymphocytes. - Modulates the activity of monocytes.	15,39,62,74
Debridement action	- Provides moist environment. - Proteases activity	- Removes dead or contaminated tissues - Removes necrotic material - Contributes to the painless lifting off of slough	58,103,104
Stimulation of tissue growth	- Stimulation of angiogenesis - Supplies glucose to provide energy and migration cells	- Hastens healing - Minimizes the need for skin grafting	55,85,108,124
Boosting of the immune system	- Macrophages - Leukocytes - Peptides and proteins - Prebiotic	- Produces cytokines, growth factors and inflammatory mediators - Collagen synthesis - Stimulates angiogenesis	1,15,57

Table 2. Demographic and treated wounds details of the honey treated and control populations.

Study	Wound Type	Treatment strategy	The number of participants	Result
72	Superficial partial-thickness burns	Honey	150	Greater efficacy of honey over SSD cream for treating superficial and partial-thickness burns.
125	Burn wound	Tualang honey	10	Tualang honey can pose substantial bactericidal as well as bacteriostatic effect. However, for gram positive bacteria, Tualang honey is not as effective as usual care products such as silver-based dressing or medical grade honey dressing.
126	Full thickness burn wound	Honey	25	Early tangential excision and skin grafting were clearly superior to expectant treatment using topical honey in patients.
127	Superficial burn wound	Honey	25	Honey early subsidence of acute inflammatory changes, better control of infection and quicker wound healing than SSD.
128	Burn wound	Honey and boiled potato peel	50	100% of the wounds treated with honey healed within 15 days while 50% of the wounds treated with boiled potato peel dressings, healed.
129	Burn wound	Honey and amniotic membrane	64	The burns treated with honey healed earlier as compared to the amniotic membrane. Residual scars were noted in 8% of patients treated with honey and in 16.6% of cases treated with amniotic membrane.
130	Burn wound	Honey and polyurethane film (OpSite)	46	Honey impregnated gauze dressed wounds showed healing earlier as compared to OpSite
131	Wound	Honey	40	There was no evidence of a real difference between honey and IntraSite Gel as healing agents. Honey is a safe, satisfying and effective healing agent. Natural honey is extremely cost effective.
132	Split-thickness skin graft donor site	Honey	88	The use of honey-impregnated gauzes is effective, safe, and practical.
87	Neuropathic diabetic foot ulcers	Manuka honey	63	Significant reduction in the time of healing and rapid disinfection of ulcers.
133	Diabetic foot ulcers	Honey	30	Ulcer healing was not significantly different in both study groups.
134	Infected diabetic foot ulcers	Honey	30	Honey decreased the size of wounds by improving organization of the granulation tissue. Reduced bacterial contamination.
135	Chronic diabetic foot ulcers	Honey	172	Significant reduction rate of amputation and improved wound healing.
136	Sloughy venous leg ulcers	Manuka honey	108	Manuka honey was effective in eradicating methicillin-resistant *Staphylococcus aureus* (MRSA) from 70% of chronic venous ulcers. The potential to prevent infection is increased when wounds are de-sloughed and MRSA is eliminated.
137	Chronic venous leg ulcers	Honey	6	The use of medical honey in patients who have received split-skin grafts for chronic venous leg ulcers appears to give a similar healing time to that seen without the application of honey.
138	Venous leg ulcers	Honey	40	Decreasing pain and size significantly, Positive impact on patient satisfaction degree with the Medi-honey treatment.

139	Venous leg ulcers	Honey	368	Honey did not significantly improve venous ulcer healing at 12 weeks compared with usual care.
140	Chronic leg ulcers	Honey and phenytoin	50	There was no significant difference in both study groups. Phenytoin may be superior to honey as a topical agent in the treatment of chronic ulcers.
141	Pressure ulcers	Honey	25	Healing rate among subjects who received honey dressing was approximately 4 times greater when compared to the control group. The use of a honey dressing is effective and practical.
142	Post-operative wound infections	Honey	50	Faster eradication of bacterial infections was seen. Honey reduced the period of antibiotic use and hospitalization, accelerated wound healing, prevented wound dehiscence and the need for re-suturing and resulted in minimal scar formation.
143	Wound by secondary intention.	Honey (Medihoney™)	105	Honey significantly reduced time of healing compared to the controls.
144	Chronic wounds	Manuka honey	20	Significant reduction in wound pH and wound size.
145	Wound	Honey (Medihoney™)	104	Honey can be an effective and feasible treatment option for professional wound care.
101	Open or infected wounds	Honey	40	Honey appears to be more effective than sugar in reducing bacterial contamination and promoting wound healing, and is slightly less painful than sugar during dressing changes and motion.
146	Chronic, complicated surgical, or acute traumatic wounds	Honey	60	Except 1 patient, in all the patients honey was easy to apply, helpful in cleaning the wounds, and had no side effects.

Figure legends

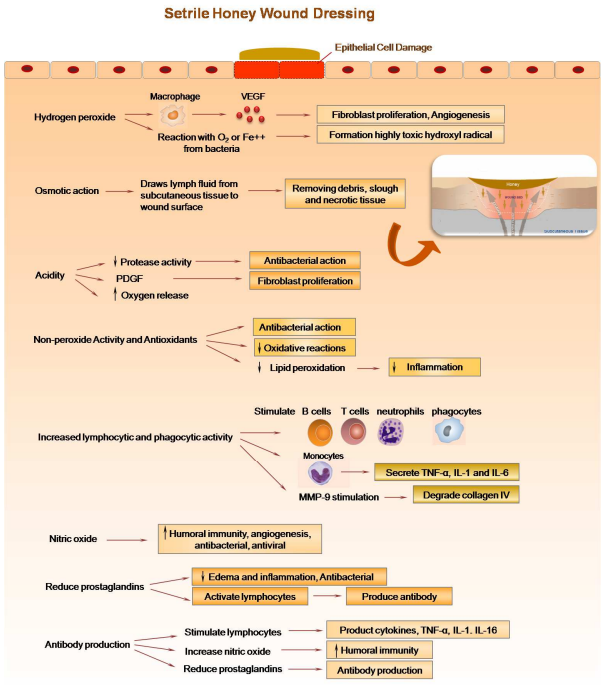
Fig 1. Mechanisms of honey during wound healing

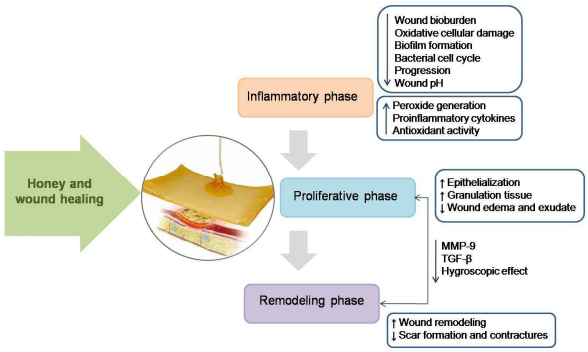
Fig 2. Modulatory effects of honey on classical phases of wound healing

Fig 3. Meta-analysis: Forest plot of the difference number of healed burn wounds between the treatment (honey) and control groups.

Fig 4. Meta-analysis: Forest plot of the difference mean of time to healing between the treatment group and the control group in burn wounds.

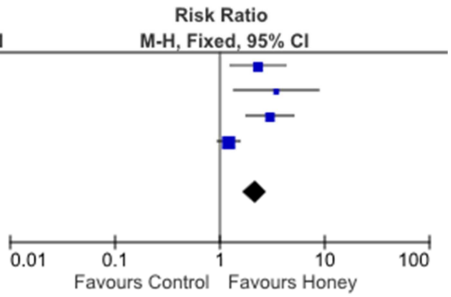
Fig 5. Meta-analysis: Forest plot of the difference number of healed chronic wounds between the treatment (honey) and control groups.





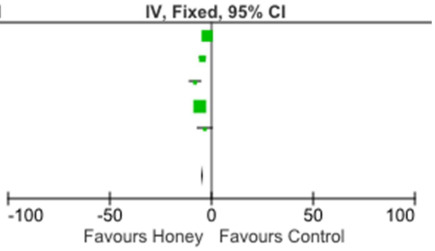
Study or Subgroup	Honey Events	Total	Control Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Malik et al. 2010	30	150	13	150	26.5%	2.31 [1.25, 4.25]
Subrahmanyam 1994	23	40	4	24	10.2%	3.45 [1.36, 8.77]
Subrahmanyam 1996	36	50	12	50	24.5%	3.00 [1.78, 5.06]
Subrahmanyam 1998	23	25	19	25	38.8%	1.21 [0.94, 1.55]
Total (95% CI)		**265**		**249**	**100.0%**	**2.17 [1.66, 2.83]**
Total events	112		48			

Heterogeneity: Chi² = 23.57, df = 3 (P < 0.0001); I² = 87%
Test for overall effect: Z = 5.73 (P < 0.00001)



Study or Subgroup	Honey Mean	Honey SD	Honey Total	Control Mean	Control SD	Control Total	Weight	Mean Difference IV, Fixed, 95% CI
Malik et al. 2010	13.47	4.06	150	15.62	4.4	150	35.4%	-2.15 [-3.11, -1.19]
Subrahmanyam 1993	10.8	3.93	46	15.3	2.98	46	16.0%	-4.50 [-5.93, -3.07]
Subrahmanyam 1994	9.4	2.52	40	17.5	6.66	24	4.2%	-8.10 [-10.88, -5.32]
Subrahmanyam 1996	10.4	2.2	50	16.2	2.3	50	41.8%	-5.80 [-6.68, -4.92]
Subrahmanyam 1998	4.92	3.61	25	8.22	8.31	25	2.6%	-3.30 [-6.85, 0.25]
Total (95% CI)			**311**			**295**	**100.0%**	**-4.33 [-4.90, -3.76]**

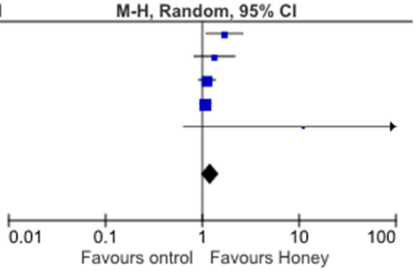
Heterogeneity: Chi² = 38.01, df = 4 (P < 0.00001); I² = 89%
Test for overall effect: Z = 14.89 (P < 0.00001)



Study or Subgroup	Honey Events	Total	Control Events	Total	Weight	Risk Ratio M-H, Random, 95% CI
Al-Waili and Saloom 1999	22	26	12	24	15.4%	1.69 [1.10, 2.61]
Gethin and Cowman 2008	24	54	18	54	13.3%	1.33 [0.82, 2.16]
Jull et al. 2008	104	187	90	181	32.4%	1.12 [0.92, 1.36]
Kamaratos et al. 2012	31	32	28	31	38.3%	1.07 [0.94, 1.22]
Yapucu Günes and Eser 2007	5	25	0	25	0.5%	11.00 [0.64, 188.95]
Total (95% CI)		**324**		**315**	**100.0%**	**1.22 [0.99, 1.50]**
Total events	186		148			

Heterogeneity: Tau² = 0.03; Chi² = 8.91, df = 4 (P = 0.06); I² = 55%

Test for overall effect: Z = 1.83 (P = 0.07)



Highlights

1. Different types of honey have different medicinal value and effects on wound healing.
2. Honey has anti-microbial, immunomodulatory, anti-oxidant and wound healing properties.
3. Honey increases wound contraction, re-epithelialization and reduces excessive scar formation.
4. Most of the clinical trials suggest the use of honey for management of various wound models.
5. Honey is a safe, cost-effective and beneficial dressing biomaterial for wound management.