Honey: an immunomodulatory agent for disorders of the skin

Pauline McLoone [1*], Mary Warnock [2], Lorna Fyfe [2]

[1] *Department of Biomedical Sciences, School of Medicine, Nazarbayev University, Astana, Kazakhstan.*

[2] *Dietetics, Nutrition and Biological Sciences, Queen Margaret University, Musselburgh, East Lothian, Scotland, United Kingdom.*

[*]Corresponding author: Dr Pauline McLoone, Department of Biomedical Sciences, School of Medicine, Nazarbayev University, Astana, Kazakhstan. E-mail: pauline.mcloone@nu.edu.kz tel. +77753651472

Abstract

Studies have shown that honeys from around the world can inhibit the growth of a range of dermatologically important microbes. As well as reports of the antimicrobial properties of honey, a number of recent *in vitro* and *in vivo* studies suggest that honey is able to modulate immunological parameters related to the skin immune system. Paradoxically, both immune-stimulatory and anti-inflammatory effects have been observed. In this review, scientific research investigating the immunomodulatory properties of honeys from around the world, in relation to disorders of the skin, is evaluated. Whilst there is sufficient evidence to suggest that honey does indeed have immunomodulatory properties, which may at least partially explain the ability of honey to promote the healing of wounds, there are still gaps in the scientific knowledge and literature. More research is necessary for a more complete understanding of the immune modulating properties of honey and to enable the utilisation of honey as an immune-modulating agent in dermatology.

Key words: honey; immunomodulatory; skin disorders; wound healing.

Introduction

Traditionally, honey has been used around the world to treat skin disorders with a microbiological and immunological aetiology. In Ayurvedic medicine, for example, honey is used to treat cuts and wounds, eczema, dermatitis, burns and Fournier's gangrene (Ediriweera & Premarathna, 2012; Deshpande & Kulkani, 2010).

In modern day clinical practice, manuka honey, produced by honey bees (*Apis mellifera*) feeding on the manuka tree (*Leptospermum scoparium*), is applied topically in the treatment of wounds (Irish, Blair & Carter, 2011). It has been approved for clinical use in countries including; New Zealand, Australia, United States of America, Canada and the United Kingdom.

A significant proportion of the world's population suffer from skin disorders and these can have a great impact on quality of life (Mahler, Jackson & Ijacu, 2009). Skin disorders have been shown to have a variety of causes; some are associated with microbial skin invasion, others with exposure to irritants e.g. chemicals in cosmetics, others are immunological in nature and result from hypersensitivity responses to environmental allergens (Fonacier, Dreskin & Leung, 2010). Current treatments for some chronic skin conditions are deficient, for example, microbial drug resistance has hindered the effectiveness of antibiotics in the treatment of chronic wounds and ultraviolet radiation therapy used to treat psoriasis and atopic dermatitis may induce skin tumourigenesis (Gasparro, 2000).

The skin healing properties of honey have been largely attributed to its anti-microbial properties; a plethora of *in vitro* studies have demonstrated antimicrobial activity of honeys from all over the world against dermatologically important microbes (Schneider, Coyle,

Warnock, Gow & Fyfe, 2013; Kuncic, Jaklic, Lapanje & Gunde-Cimerman, 2012; McLoone, Warnock & Fyfe, 2015). More recently, *in vitro* research has suggested that honeys of diverse floral origins can modulate the skin immune system; both immunostimulatory and anti-inflammatory effects have been observed (Majtan, Kumar, Majtan, Walls & Klaudiny, 2009; Majtan et al, 2013). In this review, the scientific evidence for the immunomodulatory properties of honey in relation to disorders of the skin is evaluated and the mechanisms of the immunomodulatory properties explored. A principle aim was to understand more about the therapeutic potential of honey as a treatment for immune-mediated skin disorders.

Immunomodulatory properties of honey

The skin immune system plays a major role in killing skin invading microbes yet an overstimulated and uncontrolled immune response contributes to the pathology of a variety of skin disorders. During wound healing, for example, the skin immune system plays an important role in the eradication of pathogenic microbes and tissue repair, but unregulated inflammation is an important factor associated with inefficient healing in chronic wound infections (Bowler, 2002). The skin immune system is comprised of a complex network of cells, molecules and physical barriers that prevent infection and maintain skin health. Skin cells called keratinocytes play an important function in protecting the skin from infection and also play a role in the pathophysiology of certain skin diseases (Albanesi, Scarponi, Giustizieri & Girolomoni, 2005). Keratinocytes can recognise foreign microbes via toll-like receptors expressed on their surface and release cytokines and anti-microbial peptides (AMPs) in response. Other skin residing cells, such as, macrophages, dendritic cells, mast cells and circulating T cells perform immune surveillance and neutrophils and monocytes infiltrate the skin, for example, during infection, inflammation or wound healing.

Honey or its components have been shown to modulate cytokine production by a range of cells (Majtan et al, 2013; Tonks et al, 2003). It may be that honey contributes to skin healing not only through its anti-microbial properties but by boosting the skin immune system to fight infection, promoting wound healing or suppressing inflammation in the skin. Knowledge of the immunomodulatory properties of honey would provide a greater understanding of its therapeutic mechanisms and may uncover innovative treatments for skin disorders that are immune-mediated.

Immunomodulatory properties of honey (immune cells): in vitro studies

Tonks, Cooper, Price, Molan & Jones (2001) reported that two New Zealand honeys; manuka and pasture honey significantly reduced the production of reactive oxygen intermediates (ROIs) by MM6 cells (a monocytic cell line). Pasture honey was found to be the most effective. The researchers also demonstrated that manuka and pasture honey stimulated the production of the cytokine tumour necrosis factor- alpha (TNF-α) by the same cells. The mechanisms of the honeys immunomodulatory properties were unknown but the authors suggested that high levels of hydrogen peroxide in the pasture honey may have induced a negative feedback effect on the production of ROIs by the MM6 cells. Reactive oxygen species are produced in abundance in chronic wounds and stimulate inflammation (Wlashek & Scharffetter-Kochanek, 2005). Honey could therefore be therapeutic in the treatment of chronic wounds by suppressing inflammation via the inhibition of ROI production. It was concluded that manuka and pasture honey from New Zealand have immunomodulatory properties and that these findings may at least partially explain the observed effects of honey on wound repair, particularly as TNF-α has been shown to promote macrophage activation, stimulate angiogenesis and re-epithelialisation during early wound healing (Barrientos, Stojadinovic, Golinko, Brem & Tomic-Canic, 2008).

Tonks et al (2003), further reported that Australian jelly bush honey and New Zealand manuka and pasture honey stimulated increased production of the cytokines TNF-α, IL-1β and IL-6 by MM6 cells and human blood monocytes. Australian jelly bush honey was found to be the most effective with peak cytokine levels increasing from 0 to 500 pg/ml for TNF-α, 0 to 1300 pg/ml for IL-6 and 0 to 120 pg/ml for IL-1β. The components in the honey that stimulated the upregulation of cytokine production were not identified. As the cytokines measured in the study are thought to play a role in wound healing, for example, IL-6 has been shown to enhance proliferation of keratinocytes and attract neutrophils and IL-1β stimulates the release of important wound healing growth factors, the authors suggested that this immunomodulatory effect may at least partially explain the wound healing properties of honey (Barrientos et al, 2008).

Later in 2007, Tonks *et al*, identified a 5.8kDa component via fractionation of manuka honey that stimulated TNF-α cytokine production by MM6 cells, murine bone marrow derived macrophages (MBMDM) and human monocytes. It was demonstrated that the 5.8kDa component stimulated cytokine production via toll-like receptor 4 (TLR-4) as incubation with a blocking antibody against TLR-4 blocked the immunostimulatory effect. Also, the 5.8kDa component did not stimulate cytokine production in MBMDM isolated from TLR-4 knockout mice but did for MBMDM isolated from TLR-2 knockout mice.

In the three publications by Tonks *et al*, the researchers were of the view that the immunomodulatory effects of the honey were not due to lipopolysaccharide (LPS) from contaminating bacteria, as levels in the honeys were described as low (< 1 ng/ml) in all the honeys used in their studies. Additionally, Tonks et al, (2007) showed that the immunomodulatory effects of the honey were inhibited by heat treatment; LPS is a heat stable compound and not affected by Polymixin B (PMB), a known inhibitor of LPS.

Moreover, treatment of cells with 1ng/ml LPS did not stimulate cytokine production to levels induced by the honey.

However, the role of LPS in the immunomodulatory properties of honey was to become more controversial when Timm, Bartfelt & Hansen (2008) reported their findings of an investigation into the ability of manuka honey (UMF 16+ and active 5+), Danish honey (Jakobsen & Hvam) and artificial honey to stimulate IL-6 production by MM6 cells and reactive oxygen intermediate (ROI) release from trans retinoic acid differentiated HL-60 cells (cells that are terminally differentiated along the neutrophil pathway and share similar characteristics to neutrophils). The results showed that both the manuka and the Danish honeys stimulated IL-6 production and ROI release suggesting the presence of an immunostimulatory agent in the honeys. The researchers measured the levels of LPS in the honeys and described the levels as substantial, no less than 69 pg/ml (0.069 ng/ml). They reported that the amounts of IL-6 and ROI induced by the honeys correlated with the amount that would be expected due to the content of LPS in the honey with correlation coefficients of 0.99 and 0.94 respectively. Timm et al (2008) also stated that the immunostimulatory component in the honey was resistant to boiling and could be inhibited by the addition of polymixin B. The authors were of the opinion that the immunostimulatory effects of the honey could be solely explained by the LPS content of the honeys. Confirmation of this conclusion can be obtained from the study by Moesby et al, (1999) showing that LPS levels as low as 3.1 pg/ml (0.0031ng/ml) can stimulate IL-6 production by MM6 cells (Moesby, Jensen, Hansen & Christensen 1999).

Gannabathula *et al*, (2012) reported that New Zealand kanuka, manuka and clover honeys obtained from bees feeding on *Kunzea ericoides*, *Leptospermum scoparium* and *Trifolium* species respectively stimulated TNF-α protein production by the differentiated monocytic cell lines THP-1 and U937. The cells were differentiated to macrophages with phorbol 12-

myristate 13-acetate (PMA) and TNF-α levels were measured in cell supernatant following treatment with honey by Enzyme Linked Immunosorbent Assay (ELISA). Kanuka honey was found to be the most effective honey and induced approximately 350 pg/ml TNF-α; by comparison, 100 ng/ml LPS produced TNF-α levels of approximately 500 pg/ml. TNF-α levels induced by manuka and clover honeys were less than 20% of that induced by kanuka honey. The authors were of the view that the concentrations of LPS in the honeys did not correlate with their immunostimulatory activities (kanuka honey = 2ng/ml LPS, manuka and clover honeys < 2ng/ml LPS). The addition of polymixin B completely inhibited the ability of manuka honey to stimulate TNF-α production, and reduced the ability of kanuka honey to stimulate TNF- α production by 80%. It was concluded that kanuka honey contains a major polymixin B sensitive component that is largely responsible for its immunostimulatory activity and a minor polymixin B insensitive component. Heating the kanuka honey reduced the immunostimulatory effect by 20-35% and fractionation revealed that the immunostimulatory activity was associated with a high molecular weight component (> 30kDa). The researchers had previously identified type II arabinogalactans (AGPs), known immunostimulators with a molecular weight of approximately 110 kDa, in kanuka honey. Arabinogalactans are essential polysaccharide polymers found in the cell walls and tissues of plants and can be transported to the honey from plants by bees during the honey making process. The study also demonstrated the ability of AGPs to stimulate TNF-α production by the same cell lines with levels reaching approximately 180 pg/ml with 25µg/ml AGP. The immunostimulatory effects of the AGPs were found to be polymixin B sensitive and heat stable and levels of LPS in the purified AGPs were found to be 0.31ng /5µg AGP. The authors proposed that AGPs may possess a polymixin-B binding lipid moiety enabling polymixin B to inhibit the immunostimulatory effects of AGPs. The final conclusion was that AGPs are important immunostimulatory components of kanuka honey.

Raynaud et al (2013), reported that thyme honey provided by Melipharm® (Limoges, France) stimulated PGE_2, COX-2 and TNF-α protein production and also induced NFκB and AP-1 activation in RAW 264.7 murine macrophages. The authors concluded that the effects of the honey could not be explained by the LPS content as the levels of LPS in the honey were insufficient to stimulate the production of these molecules to levels induced by the honey. Macrophages play an important role in the wound healing process by phagocytosing foreign material and debris in the wound bed and by producing cytokines that stimulate tissue repair (Nguyen, Orgill & Murphy, 2009). The authors proposed that honey induced activation of NF-κB and AP-1 could induce COX-2 production leading to increased PGE_2 production. AP-1 regulates gene expression of various inflammatory mediators such as inducible nitric oxide synthase, TNF-α and IL-1β. Honey induced upregulation of early inflammatory mediators, via AP-1 or NF-κB activation, may promote the early wound healing process.

It may also be of interest to note the study of Abuharfeil, Aloran & Aboshehada (1999) reporting that honey produced by honey bees (*Apis melifera*) kept in a hive at Jordan University Campus farm, Jordan, stimulated the proliferation of unstimulated human B and T lymphocytes collected from human blood as measured by an MTT assay. The researchers concluded that their honey contains unidentified lymphomitogens and suggested that these lymphomitogens could be plant lectins obtained from plants during the honey making process. In relating this work to the skin it may be that honey is able to stimulate the proliferation of skin residing T cells although further research would be required to investigate this.

Other *in vitro* studies have also shown anti-inflammatory effects of honey on immune cells. Ahmad, Khan & Mesaik (1999) reported that six different types of commercial honey (clover honey from USA, capilano from Australia, langanese from Germany, al-shafa, swat and sidder from Pakistan) purchased in a supermarket in Pakistan inhibited bovine thrombin

induced respiratory burst in human neutrophils and rodent peritoneal macrophages. In line with this, Henriques, Jackson, Cooper & Burton (2006) observed free radical quenching activity in three honeys of different origin (gales honey obtained from a supermarket in the UK and pasture and manuka honey obtained from New Zealand). These findings suggest that honey may be able to reduce inflammation by quenching free radicals in an inflammatory site. Chepulis and Francis, (2012) reported that raw manuka honey containing high levels of Methylglyoxal (MGO) obtained from bee keepers in the Wairapa region of New Zealand and supplied by Manuka Health New Zealand Ltd had contradictory effects on TNF-α production by neutrophils. Both the MGO250^{TM} and MGO400^{TM} manuka honey samples induced inhibition of TNF-α production by neutrophils when measured at honey concentrations of 400µg/ml. Honey concentrations of 100µg/ml MGO250^{TM} stimulated TNF-α production by neutrophils (see table 1).

Immunomodulatory properties of honey (skin cells): in vitro studies

In 2009, Majtan et al reported the ability of acacia honey obtained from Slovakia to stimulate TNF-α, TGF-β, IL-1β and matrix metalloproteinase 9 (MMP-9) mRNA expression by human primary keratinocytes isolated from human foreskin. TNF-α mRNA expression was up-regulated two-fold by the honey and TGF-β and IL-1β mRNA expression were up-regulated eight-fold and ten-fold respectively. This stimulation was not thought to be due to LPS as LPS levels in the honey were found to be 0.42 ng/ml and 10 ng/ml of lipopolysaccharide did not stimulate cytokine mRNA expression in these cells. The researchers also investigated the ability of the honey component major royal jelly protein 1 to stimulate cytokine production by the same cells and found there was a significant increase in TNF-α mRNA expression and a non-significant increase in levels of TGF-β and IL-1β mRNA. The authors suggested that because keratinocytes play an important role in the production of cytokines and MMP-9

during early wound healing, stimulation of such molecules by honey may aid the wound healing process.

Contrary to the immunostimulatory effects of honey described above Majtan et al (2013) reported an anti-inflammatory effect of an aqueous extract of fir honeydew honey obtained from an apiary in Bardejov, Slovakia. In this study, the extract inhibited TNFα induced matrix metalloproteinase-9 (MMP-9) protein and mRNA production by human keratinocytes (HaCaT) cells as measured by ELISA and RT-PCR respectively. The phenolic compounds kaempferol and apeginin were identified in the honey and reduced TNF-α induced MMP-9 production in a dose dependent manner. MMP-9 is thought to play an important yet temporary role in normal wound healing through its involvement in re-epithelialisation and extra-cellular matrix remodelling. However, in chronic wounds it is found that MMP-9 levels remain persistently elevated and is thought to be responsible for the degradation of matrix and cell growth promoting agents in chronic wounds (Widgerow, 2011). The researchers concluded that the aqueous extract and flavonoids from fir honey dew honey are anti-inflammatory and their ability to suppress TNFα induced MMP-9 production by human keratinocytes may explain the therapeutic effects of honey in the treatment of chronic wounds.

Ranzato, Martinotti & Burlando (2013) reported that acacia and buckwheat honey (purchased from an apiculture centre in Japan) upregulated IL-4 and IL-8 production and decreased MMP production in human dermal fibroblasts. These honeys were also shown to have chemoattractant properties and stimulated wound repair of human dermal fibroblasts *in vitro*.

Several studies have reported that ability of honey to promote faster re-epithelialisation rates in skin cells *in vitro* (Ranzato, Martinotti & Burlando (2012); Barui et al, 2013).

Immunomodulatory properties of honey: in vivo studies

To the best of our knowledge there are no studies to date investigating the effects of honey specifically on the skin immune system of humans *in vivo* but such studies are recommended. Some suggestions would be to investigate the effects of honey on contact hypersensitivity or delayed type hypersensitivity responses induced in humans *in vivo*. There are studies investigating the efficacy of honey in the treatment of skin disorders including wounds, of which the effects may be caused by the *in vivo* immunomodulatory properties of honey (Naidoo *et al*, 2011; Jull *et al*, 2015). For example, a honey mixture (containing honey, beeswax and olive oil) was found to significantly improve the symptoms of atopic dermatitis, seborrheic dermatitis, psoriasis, pityriasis versicolor and tinea cruris (Al-Waili, 2001; 2003; 2004).

An animal study worthy of note is that by Choi et al (2014) which provided evidence that a topical herbal honey and chitosan cream formulation reduced symptoms in atopic dermatitis like lesions induced *in vivo* in mice. The lesions were induced with 2, 4 dinitrochlorobenzene (DNCB), a contact sensitiser. The honey was obtained from hives in Mount Jiri, Korea. Serum IgE, IL-4 and IL-12 levels as well as dermal mast cell infiltration were also reduced in the honey/chitosan treated group.

Discussion

It can be concluded from the aforementioned *in vitro* research that honeys from a variety of origins have immunomodulatory properties. These effects have been shown to be both immunostimulatory and anti-inflammatory and such opposing results may be due to the complex nature of the composition of different kinds of honey and also to differences in cell types used and parameters measured across studies. The scientific evidence suggests that honey can stimulate the production of immunological mediators such as cytokines that play

an important role in the early wound healing process and downregulate the production of molecules e.g. MMPs and ROIs that contribute to excessive inflammation in the chronic wound (see Figure 1). In a review paper by Majtan (2014) the author speculates that honey stimulates cytokine and MMP production from keratinocytes when there is a low level of inflammation e.g. in a non-infected wound. When a wound is infected and there is a high level of inflammation the authors suggest that honey suppresses the production of inflammatory cytokines and MMPs by keratinocytes. In the majority of studies, researchers have made extrapolations from their findings in relation to honey as a treatment for wounds, most likely because of the clinical use of honey in wound healing today. There are, however, other skin disorders with an immunological aetiology e.g. atopic dermatitis and psoriasis that respond to topical immunomodulating agents such as steroids and immunosuppressive UV therapy. As research has identified honey as an immune modulating agent, it is logical to consider honey as a potential therapeutic agent for other immune mediated skin disorders. However, due to the complex nature of the immune system in skin disease, it may be difficult to infer what effects honey will have on such conditions without further investigation. The cytokines TNF-α, IL-18 and IL-8 are pro-inflammatory cytokines that contribute to the pathology of inflammatory skin disorders, whilst IL-10 on the other hand, is an immunosuppressive cytokine capable of down-regulating inflammation (Numerof and Asadullah, 2006; Noh and Lee, 2012). TNF-α, IL-18 and IL-8 have also been shown to play a role in wound healing (Kanno et al, 2013; Kampfer et al, 2000; Jiang, Sanders, Ruge & Harding, 2012). An investigation into the ability of honey to modulate the production of such cytokines by skin cells would help determine if honey has immunosuppressive, immune stimulatory or wound healing properties that may contribute to skin healing. Antimicrobial peptides (AMPs) such as Cathelicidin LL-37 and hBD-2 not only function as antimicrobials protecting skin from pathogenic microbes such as *Staphylococcus aureus* but also as

immunomodulators (Li, Xiang, Zhang, Huang & Su, 2012). LL-37 has also been shown to contribute to cutaneous wound healing by stimulating re-epithelialization (Heilborn et al, 2003). An investigation into the ability of honey to modulate AMP production would determine if honey is able to affect wound healing or the skin immune response against pathogenic microbes. Additionally, the production of AMPs is thought to be perturbed in certain inflammatory skin disorders; increased in psoriasis and decreased in eczema (Nakatsuji and Gallo, 2012). The ability of honey to modulate AMP production may contribute to skin homeostasis in such skin disorders. The immunomodulatory properties of honey may also have relevance in the fight against skin cancer, particularly as the immune system plays an important role in preventing the outgrowth of transformed tumours.

The components in honey responsible for its *in vitro* immunomodulatory properties have not been fully identified but have been attributed to lipopolysaccharide, a 5.8kDa component, major royal jelly protein 1, arabinogalactans, polyphenols and antioxidants. Production of reactive oxygen intermediates (ROIs) are an important cause of excessive inflammation because they can induce oxidative activation of NF-κB, leading to the production of pro-inflammatory mediators (Novo and Parola, 2012). It has been proposed that antioxidants can reduce inflammation via inhibition of NFκB activation. Chepulis and Francis (2012) however did not find a direct correlation with the total antioxidant levels and anti-inflammatory results although antioxidant levels were found to be high in the honeys they tested. It therefore may be that antioxidants are not the sole anti-inflammatory agents present in honey.

The immunomodulatory properties of honey are complex and not yet fully understood. Despite the interesting findings to date, it is evident that further research is necessary for a

fuller understanding of the ability of honey to modulate the skin immune system, particularly *in vivo,* and to establish how such properties may be utilised clinically for the amelioration of skin disease. As well as the complex nature of honey itself, it is likely that the microenvironment of the skin disorder will have a major influence on the efficacy of honey in clinical practice. A more complete identification of the immunomodulatory components in honey may be useful for the development of novel immunotherapeutic agents. It may be that honey exerts synergistic therapeutic effects when combined with other agents and this would be an area worth exploring. As with the antimicrobial properties of honey, innovative research that can maximally exploit the immunomodulatory properties of honey may in the future lead to the manufacturing of a product that is highly valued in dermatology. Also, with the wide variety of honeys being produced worldwide it may be that some have superior immune modulating properties that are yet to be discovered. As our scientific knowledge of honey advances it seems that the properties of this natural substance are becoming more and more remarkable.

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Table 1. Percentage inhibition of TNF-α production by neutrophils incubated with manuka honey samples with different methylglyoxal (MGO) content. Manuka honey samples were obtained from Waiarapa region of New Zealand. (Results from Chepulis & Francis, 2012).

Manuka Honey Sample	% Inhibition of Production of Tumour Necrosis Factor-alpha (TNF-α) by Neutrophils
MGO 250™ 100 μg/ml	-30.2%
MGO 250™ 400 μg/ml	+29.9%
MGO 400™ 100 μg/ml	+15.3%
MGO 400™ 400 μg/ml	+19.5%

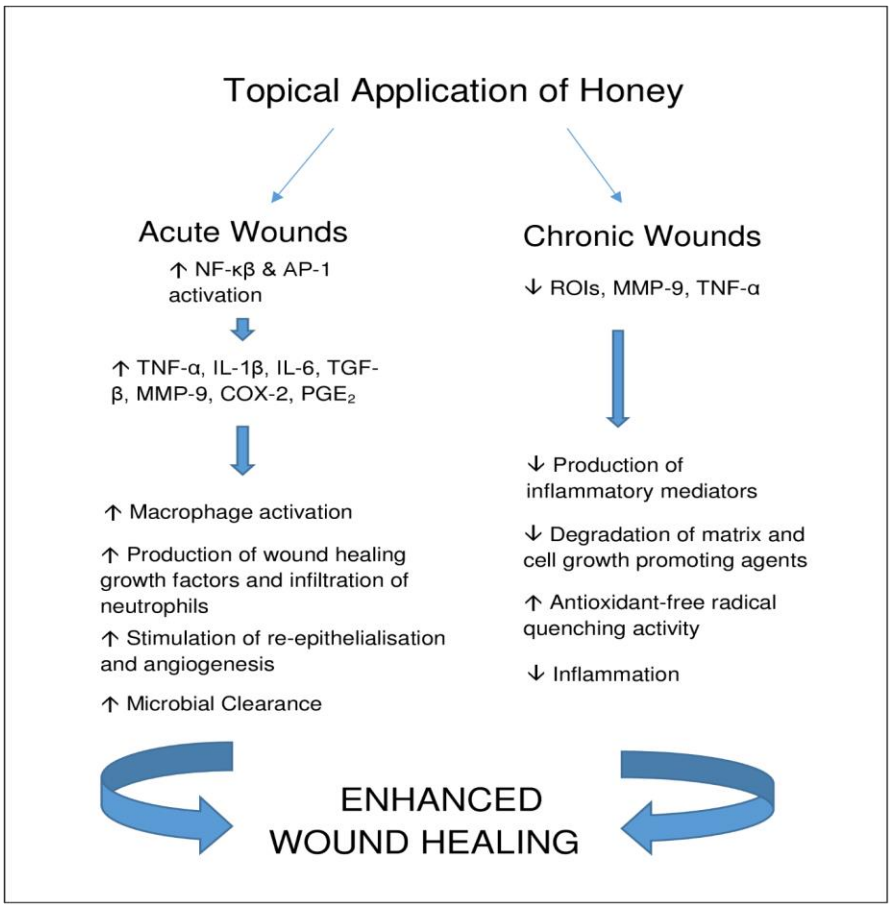

Figure 1. Possible mechanisms by which the immunomodulatory properties of honey could enhance the healing of both acute and chronic wounds. (↑ - increased, ↓- reduced).