

# What's the buzz: bee products and their potential value in diabetic wound healing.

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The Journal of Diabetic Foot Complications, 2014; Volume 6, Issue 2, No. 1, Pages 24-39 © All rights reserved.

## Abstract:

Foot ulceration, secondary to diabetes, is the most common reason for lower limb amputation, accounting for 50-70% of non-traumatic lower limb amputations. Rather than progressing through the usual wound healing phases, diabetic wounds become 'stuck', predominantly in the inflammatory phase. Normal feedback mechanisms that conclude the inflammatory stage are short-circuited, and the inflammatory response is upregulated and persistent. Chronic diabetic wounds always have a bacterial load, and the increased tissue bacterial burden may impede healing. Since ancient times, bee-derived products have been used as medicines and as potential wound healing therapies. Their anti-inflammatory and anti-bacterial properties have been widely reported. Honey, propolis, royal jelly, and bee venom have pre-clinical wound healing properties. This review seeks to examine factors that prevent diabetic wound healing and the potential of four bee products to promote diabetic human healing in these wounds. The indication for key clinical trials in this exciting area of bee-derived products is also emphasized.

**Key words:** Apitherapy, Bee Venom, Diabetic Foot Ulcer, Honey, Propolis, Royal Jelly

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### Abbreviations

DFU	Diabetic Foot Ulcer
ECM	Extra-Cellular Matrix
MMP	Matrix Metallo-Proteinase
AGE	Advanced Glycation End-product
RAGE	Receptor for Advanced Glycation End-product
TNF $\alpha$	Tumor Necrosis Factor - Alpha

MRJP	Major Royal Jelly Protein
TIMP	Tissue Inhibitor of Metallo-Proteinases
CAPE	Caffeic Acid Phenyl Ester
NF - $\kappa$ B	Nuclear Factor Kappa -Beta
ROS	Reactive Oxygen Species
MRSA	Methicillin-resistant Staphylococcus aureus
MGO	Methylglyoxal
RCT	Randomized Controlled Trial

## INTRODUCTION

Foot ulcers and infections cause major morbidity for individuals with diabetes mellitus. Recognized for some decades, wounds in diabetic patients are prone to abnormal healing.<sup>1</sup>

Presently, because of diabetes about one amputation occurs globally every 30 seconds. This results in over 2,500 lower limbs being lost per day.<sup>2</sup> Diabetes is the leading cause of non-trau-

matic lower-extremity amputations in the United States (US).<sup>3</sup> For individuals with diabetes, the lifetime probability of developing a diabetic foot ulcer (DFU) is estimated at 10 to 25% (Centers for Disease Control and Prevention, 2011). In the developed world, for people with diabetes having a foot ulcer is a leading cause of hospitalizations and is a major, but often unrecognized cause of morbidity associated with diabetes. It leads to suffering and a reduced quality of life for patients.<sup>4</sup> Studies by Cavanagh et al., have shown that the costs of treatment for the patient are considerable. For example, the average cost of treatment of a DFU is the equivalent of six days of average income in the US and 5.7 years of average annual income in India. Although these findings do not take cost-effectiveness into account, they highlight the dramatic economic burden of a DFU.<sup>5</sup> Australian data from the 2000 Fremantle Diabetes Study shows that the average length of hospital stay for a DFU admission was 31 days and the cost was \$17,089 AUD.<sup>6</sup> During the study period, this represented 9.3% of the total cost of diabetic hospitalizations.

Many factors contribute to wound healing deficiencies in DFUs. These include: 1) a decrease or impairment of growth factor production or concentration<sup>7</sup>, 2) an imbalance between the accumulation of extra cellular matrix (ECM) components and their remodeling by matrix metallo-proteinases (MMPs)<sup>8</sup>, and 3) impairment of macrophage activation and angiogenesis and/or collagen synthesis, which consequently result in wounds that heal slowly and incompletely.<sup>9,10</sup> Thus, both the 'soil' (metabolic environment and wound bed status) and the 'seed' (cells involved in wound healing) appear to be impaired in diabetic wounds. Additionally, the presence or persistence of bacteria is associated with tissue destruction, and it is well-known to all who treat patients with DFUs that a high bacterial load may contribute to impaired wound healing.

The cornerstone of care in DFUs is based on a careful assessment of the nature of the foot ulcer. These include precipitating and predisposing factors, and four treatment elements, which are debridement and dressings, pressure offload-

ing, antibiotic therapy, and where indicated, revascularisation. It is the individualization of such treatments to a specific patient in a balanced manner across the multiple health care disciplines that is key in optimizing foot ulcer healing in diabetes.<sup>11</sup> Podiatrists (the treating foot care physician), vascular and foot surgeons, nurses, and broader disciplines, such as microbiologists and radiologists, all need to work together to realize the gold standard in outcomes for ulcer healing and to minimize hospital admissions and amputations.

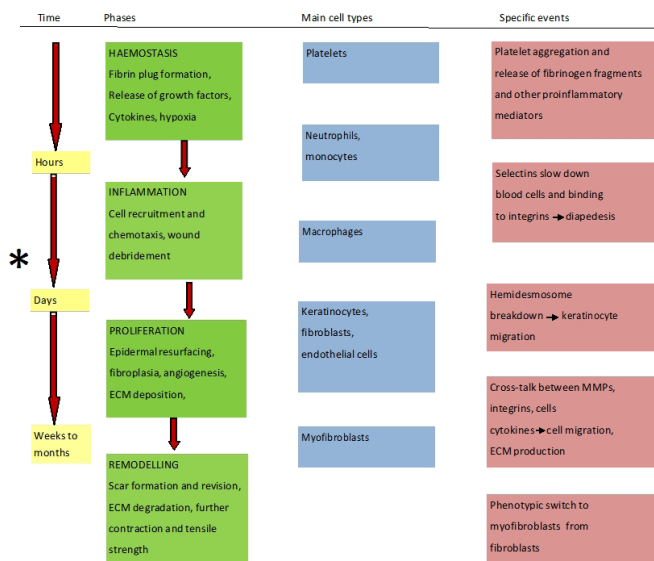
With good care from a multidisciplinary team most DFUs will heal. However, it is unfortunate that some diabetic wounds will not. Currently, the most common therapies used for DFUs are debridement and topical dressings (i.e., foams, hydrogels, nano-crystalline silver, povidone iodine), antibiotic therapy, revascularization, and pressure reduction using a total contact cast or other device rendered irremovable. Newer therapies such as topical negative pressure therapy and hyperbaric oxygen therapy<sup>12</sup> are generally expensive and often lack in supporting evidence (such as autologous platelet transfer<sup>13</sup>). These factors have precluded such treatments from becoming a mainstay of DFU treatment.

To improve healing rates in recalcitrant diabetic wounds in a cost-effective manner, researchers are looking at inexpensive and readily available natural products. The relative inexpense of developing existing natural products or 'neutraceuticals' into wound healing therapies is particularly attractive because these therapies generally have low side effects. Since ancient times bee-derived products have been used as medicines and wound healing therapies. Recently there has been a revival of interest in bee-derived products, but to date the collective role these products play in diabetic wound healing has not been studied. Bee, or 'Apitherapy' products, are of particular interest as they have anti-bacterial and anti-inflammatory properties, are inexpensive, and are generally well tolerated. This review seeks to investigate the functional properties of major bee-derived products and their potential to heal wounds in the diabetic foot.

## THE STUDY

### The Normal Wound Healing Process

Normal cutaneous wound healing is the intricate process of the skin repairing itself following an injury. This process is highly organized and occurs as a series of well-ordered, overlapping events: haemostasis, inflammation, proliferation, and remodelling. Healing in the skin (**Figure 1**), and the approximate phase where delay in DFU healing occurs, is indicated by the asterisk (\*). Wound healing is a multicellular process that is completed with both barrier restoration and functional recovery of skin strength.<sup>14</sup> At the cellular level, it involves multiple cell types with varying concentrations of cytokines, growth factors, and ECM expressed across various healing stages.<sup>15</sup>



**Figure 1.** A schematic diagram showing, following 'haemostasis', the three main stages of wound healing as it occurs in humans. The time course reflects healing by secondary intention in a cutaneous wound, with re-epithelialisation occurring in the later proliferative phase. The blue arrow indicates, as described in the text, the inflammatory phase that tends to persist in diabetic foot ulcers. Adapted from Falanga.<sup>10</sup>

### Characteristics of Diabetic Foot Ulcers

The typical DFU is characterised by its chronicity, early and persistent inflammatory phase, delayed granulation production, and reduced ability to heal. Diabetic wounds also

have a higher incidence of infection.<sup>16</sup> Currently, a comprehensive understanding of diabetic wound healing has yet to be realized.<sup>10,17</sup> However, many important factors disrupt healing in people with diabetes.

Delayed healing is attributed to a variety of factors, including micro- and macrovascular disease, neuropathy, bacterial infection, local pressure due to foot deformity, and the adverse local metabolic environment caused by diabetes. Furthermore, pathogenic factors are observed intrinsically within the wound microenvironment. These are excessive inflammation, fibronectin deficiency, fibrin cuff accumulation, leucocyte impairment (namely persistence of neutrophils and decrease in macrophage activity), MMP persistence,<sup>18</sup> accumulation of advanced glycation end products (AGEs),<sup>19</sup> abnormalities in growth factors, and other wound inflammatory mediators such as toll-like receptors (TLRs), tissue necrosis factor alpha (TNF $\alpha$ ) and Dipeptidyl Peptidase -4 (DPP-4).<sup>20,21,22</sup>

Chronic ulcers typically feature heavy bacterial colonisation.<sup>23</sup> Robson et al.<sup>24</sup> report that regardless of tissue type, any type of bacteria present at a level equal to or greater than  $10^6$  organisms/g tissue will suffer from healing impairment. Additionally, our studies have shown that healing of DFUs are impaired even when clinically significant levels of bacteria are not detected.<sup>25</sup> Whether this increased bacterial burden is a consequence of the longevity of the wound, local wound hypoxic conditions, or as a direct result of hyperglycaemia or its derivatives such as advanced glycation end-products in diabetes is uncertain. Thus, infection tends to be regarded as a complication of diabetic ulcers and a factor that secondarily exacerbates them, as opposed to playing a primary causal role.<sup>24</sup>

### Bee Products – Potential Wound Healing Therapies

Bees nest in colonies that are headed by a single fertile female, the queen, who is usually the only egg layer in the colony. Worker bees are also female. They forage nectar to make honey, produce royal jelly to feed the queen and larvae,

clean, remove debris from the hive, and produce the resinous substance called propolis which protects the hive from pathogens. These clearly defined functions enable the bee to survive a variety of evolutionary challenges. Numerous 'Apitherapy' products are produced by bees and can be harvested from their nests (**Figure 2**). Of these products, only honey, propolis, royal jelly, and bee venom have been researched for their potential as wound healing therapies. For this reason, these four products are the focus of this review.

with some subspecies being better suited to producing certain bee products than others. For example, the Russian honey bee yields more propolis than the Irish honeybee. However, most colonies can produce one of each of these products. The principal components of the various bee products are shown in **Table 1**. They vary quite markedly in composition, including in water and carbohydrate content.

Table 1. Main composition of the four bee products

Bee Product	Composition
Honey	20% water, 70–75% reducing sugars, 5-10% sucrose 125
Royal Jelly	60% water, 5% lipids, 15% protein, 20% sugars 126
Propolis	50% resin and vegetable balsam, 30% wax, 10% essential oils, 5% pollen and 5% organic debris 127, 128
Bee Venom	88% water, 6% melittin, 6% combination of other enzymes and amino acids, carbohydrates, phospholipids and physiologically active amines 129

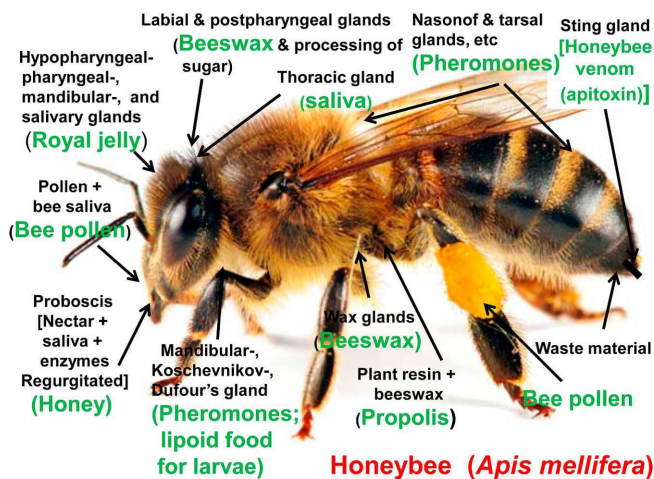


Figure 2. Secretions of the honeybee, Israili et al., adapted from American Journal of Therapeutics (2013) www.americantherapeutics.com.<sup>33</sup>

The composition of bee products shows a large amount of regional variation, based on the local species of bee, plants that they feed on, and climatic and environmental conditions.<sup>26,27</sup> The most well-studied and commonly used bee products are derived from the honey bee (*Apis mellifera*), which is native to Europe, Africa, and Western Asia. Starting in the 17th century, this species has also been found around the world, including East Asia, Australia, and North and South America.<sup>28</sup>

This species of honey bee has several subspecies, or regional varieties, including the Italian bee (*Apis mellifera ligustica*), European dark bee (*Apis mellifera mellifera*), and the Carniolan honey bee (*Apis mellifera carnica*). Each species' ability to produce multiple bee products differs,

### Honey

Honey is a viscous sugar solution derived from nectar and modified by the *Apis mellifera* honey bee. Since ancient times it has been used for wound healing. The use of honey in wound healing was documented in the hieroglyphic text 'The Edwin Smith Papyrus', dated between 2600 and 2200 BC.<sup>29</sup>

Honey composition has regional variation, determined by floral source and climate.<sup>30,31</sup> While honey composition is variable due to regional variation in bee foodstuffs, the baseline composition of the elements remains relatively static. Honey contains over 400 compounds.<sup>32</sup> Of interest in wound healing, and discussed in more detail later, are its antioxidant, anti-inflammatory, anti-microbial, and immunostimulant properties.<sup>33,34</sup> Factors contributing to antimicrobial activity of honey are high sugar concentration, hydrogen peroxide, methylglyoxal (MGO), the

antimicrobial peptide bee defensin-1, and low pH. To preserve these properties, medical grade honey must be sterilized by gamma irradiation and not heat.<sup>35</sup>

### Propolis

Bees produce a resinous hive protectant called propolis. The name derives from the Greek words Pro (defense of) and Polis (city) and reflects the importance of this substance as a hive protectant. Propolis consists of plant buds that are collected on the hind legs of worker bees. The buds are then masticated and mixed with salivary enzymes and wax.<sup>36</sup> Within the hive, the bee uses propolis to fill cracks and crevices to prevent insect invasion.<sup>37</sup> It is also used to 'embalm' hive invaders, which the bees are able to kill, but cannot transport out of the hive. This prevents problems associated with decomposition.<sup>36</sup> The composition of propolis is complex and, like honey, is subject to regional variation. It contains wax, resin, and a small amount of other compounds. Most of the 200 biologically active constituents of propolis<sup>38</sup> are contained within the resin. The most biologically active fractions of propolis are flavonoids and esters of caffeic acid<sup>39,40</sup>, but recent studies have identified other promising compounds.<sup>41</sup>

Propolis is collected by scraping down the frames of the beehive, usually in winter when the bees are less active. The extract can then be prepared a number of ways. The most common are ethanol (ethyl alcohol), ether, glycol, or water. Irrespective of extraction method, the solution is filtered to remove the debris. It can then be diluted or mixed to form a paste or gel. The most common forms are the ethanolic and water extracts, which contain water or alcohol soluble bactericidal components.

### Royal Jelly

Royal jelly is a substance of complex chemical structure produced by the young nurse bees as larval food. It is a secretion from glands on the top of their heads. It is harvested from movable frames where it has been deposited by the queen into individual queen cells. Royal jelly consists of a mixture of sugars, lipids, vitamins,

and proteins secreted from the mandibular and hypopharyngeal glands of worker bees.<sup>42</sup> Significant amounts of bioactive substances such as unsaturated fatty acids of 10-hydroxy-2-decenoic (10H2DA), 3,10- dihydroxydecanoic, and several insulin like peptides and sebatic acids<sup>43,44</sup> are found in royal jelly. Like honey and propolis, royal jelly contains phenolic compounds that are powerfully anti-oxidant.<sup>45</sup>

Many physiological functions of the royal jelly protein have been widely reported, and they possess several pharmacological activities in experimental animals. These activities are immuno-modulatory, anti-inflammatory, anti-bacterial, and antioxidative.<sup>46,47</sup> Royal jelly contains identical major proteins belonging to one protein family designated MRJP (from Major Royal Jelly Proteins). The family consists of five main members (MRJP1, MRJP2, MRJP3, MRJP4, and MRJP5). The proteins MRJP3 and MRJP5 are polymorphic. MRJPs account for 82% to 90% of total larval jelly protein, and they contain a relatively high amount of essential amino acids that play an important role in honey bee nutrition.<sup>48</sup>

### Bee Venom

Honey bee venom is produced in the abdomen of the bee from a mixture of secretions stored in the venom sac.<sup>49</sup> When administered as a sting, it causes local inflammation, therefore, deterring predators. Bee venom also acts as an anticoagulant and releases pheromones, indicating a signaling function.<sup>50</sup> Bee venom has been used in wound healing for centuries. Bee venom is collected by electrically stunning the bees to remove the venom and is known for its anti-inflammatory effects. It consists of a complex mixture of peptides such as melittin, phospholipase A2, histamine, hyaluronidase, catecholamine, and serotonin.<sup>51</sup> Melittin is the principal toxic component in the venom of the European honey bee and constitutes 50% of its dry weight.<sup>52</sup>

### Biological Activities of Bee Products

Chronic foot ulcers in persons with diabetes is, as previously discussed, due to several inter-related factors that cause local wound related ab-

normalities.<sup>53</sup> Some factors that prevent diabetic foot wounds from healing, including persistent inflammation and increased bacterial burden, can possibly be ameliorated with 'Apitherapy'/bee products.

The biological activities of honey, propolis, royal jelly, and bee venom are largely attributed to their phenolic compounds. Phenolic compounds exhibit a wide range of biological activities and contain one or more aromatic rings bearing one or more hydroxyl groups. They are categorized by the number of phenolic rings and the structural elements that link these rings.<sup>54</sup> These compounds can regulate cell signaling pathways and can lead to cell proliferation and migration, as well as cell survival.<sup>54</sup> Additionally, increased expression of anti-inflammatory genes and inhibition of MMP activities (particularly MMP-9) have been described.<sup>55</sup> Phenols also have antibacterial, antifungal, antiviral, antimutagenic, and anti-inflammatory functions. The main groups of phenols in honey, propolis, and royal jelly are the flavonoids and, as discussed, regional variation within these compounds occurs from plant, climatic, and environmental factors.

### Inflammation

Chronic foot ulcers are often stalled in the inflammatory phase. They have impaired granulation tissue formation, persistent inflammatory cell infiltrate, and decreased ECM accumulation from either increased degradation or decreased production. Together, this dysregulation of the wound healing process leads to a delay in the proliferative stage of wound healing.

Deficient peak leucocyte numbers are noted in diabetic wounds; this is attributed to defective chemotaxis and inhibited proliferation.<sup>56</sup> Phenotypic changes to various leukocytes contribute to impaired wound healing, though the mechanisms by which these occur are largely unknown.<sup>57</sup> In normal wounds, neutrophils are rapidly induced into the wound and are then scarce after 72 hours. However, neutrophils persist in diabetic wounds thus prolonging the inflammatory state.<sup>58</sup>

Excess neutrophils, combined with decreased transforming growth factor beta

(TGF- $\beta$ ) and insulin-like growth factor (IGF-1), leads to a persistent inflammatory wound environment, with the accumulation of excessive amounts of MMPs, especially MMP-1 and MMP-9.<sup>59</sup> These MMPs are not matched in quantity by their regulators, the tissue inhibitors of metalloproteinases (TIMPs), and as a result of this inflamed, protease-enhanced environment uninhibited tissue degradation occurs. Data from our laboratory shows that high levels of pro- and active MMP-9, and a high MMP-9 to TIMP-1 molar ratio in post-debridement wound fluid can predict future poor wound healing outcomes.<sup>18</sup> High circulating glucose concentrations also increase MMP-9, but the effect of this on wound fluid MMP-9 concentration is not known.<sup>60</sup> The ability of macrophages to phagocytize neutrophils, a key landmark in the conclusion of the inflammatory stage, is impaired in diabetic mice.<sup>61</sup> This deficit is attributed to decreased macrophage numbers, increased levels of apoptotic neutrophils, and increased presence of pro-inflammatory mediators. The phagocytosis of neutrophils also cues macrophages to reprogramme themselves from an inflammatory phenotype to a reparative phenotype. Lack of reprogramming can exacerbate the inflammation, thereby potentiating the cycle.<sup>62</sup>

Advanced glycation end products, or AGEs, can cause cellular ageing and also have a range of detrimental effects on wound healing.<sup>19</sup> AGEs are formed through a series of nonenzymatic reactions, mainly between glucose and proteins. Accumulation of AGE proteins in diabetic wounds is directly proportional to glycaemia and high glucose levels, indirectly prolonging inflammation. The mechanisms by which AGE damage may occur include structural modification of proteins and stimulation of cellular responses via AGE receptors.<sup>19</sup> AGEs may both bind to ECM and modify its function, or signal through cell surface receptors such as the receptor for AGEs, known as RAGE, commonly to induce cellular oxidant stress pathways.<sup>19</sup> On the basis of preclinical and clinical data, dysregulation of cytokines, MMPs, growth factor expression and inflammatory cell infiltration, and function in the diabetic wound

microenvironment can be attributed to AGE activity.<sup>19</sup>

Honey has a low pH (3-4) that not only inhibits bacterial proliferation but speeds up healing through acidification. Low pH also suppresses protease activity; a neutral pH is optimal for proteases to degrade the wound matrix.<sup>63</sup> Low pH also increases the amount of oxygen off-loaded from hemoglobin in the capillaries that reduce wound hypoxia.<sup>64</sup> The anti-inflammatory action of honey also reduces edema and the quantity of exudate by down regulating the chronic inflammatory cycle. In studies by Majtan using keratinocytes, after incubation with honey elevated production of mediators including cytokines (TNF- $\alpha$  and TGF- $\beta$ ) and MMP-9 was shown.<sup>65</sup> Whether this observation can be transferred to the complex wound healing environment with positive or negative effect is not clear, and it is likely dependent on the phase of the wound healing process.

The anti-inflammatory effects of propolis are well-established.<sup>66,67</sup> These properties are largely attributable to caffeic acid.<sup>68,69</sup> Studies by Jin et al.<sup>70</sup> showed that caffeic acid phenyl ester (CAPE) in propolis is a potent inhibitor of MMP-9. Temiz et al.<sup>71</sup> hypothesised that propolis treated rat colon anastomoses healed more quickly and showed increased bursting strength due to decreased collagenolysis attributable to CAPE action on MMP-9. Our laboratory was the first to report the use of topical propolis in a diabetic animal model of wound healing. McLennan et al. showed that propolis treatment reduced the persistent inflammation that characterizes diabetic wounds by normalizing neutrophil and neutrophil elastase counts. McLennan et al. proposed that the widely reported anti-oxidant effects of propolis<sup>73,74,75</sup> normalized inflammatory exudates in diabetic rodents and aided the observed improvement in wound closure rate in diabetic wounds.

Reduced inflammatory cell activity was observed in propolis treated rabbit eye corneal injuries compared to controls. This anti-inflammatory effect was comparable to the anti-inflam-

matory effect of dexamethasone.<sup>76</sup> Mice treated with propolis (200mg/kg) showed an inhibition of interleukins produced by spleen cells. This demonstrated the potential of propolis to reduce the chronic inflammation that characterizes many autoimmune diseases.<sup>77</sup>

The major fatty acid component of royal jelly, 10H2DA, has collagen synthetic and MMP-inhibitory activities.<sup>78</sup> Royal jelly protein prolonged the cell proliferation of primary cultured rat hepatocytes,<sup>79</sup> enhanced the migration of cultured human dermal fibroblasts,<sup>80</sup> improved granulation tissue formation, and improved dermal wound healing rate in diabetic rats.<sup>78</sup>

Bee venom constituents demonstrate anti-inflammatory properties, and it is traditionally used in many inflammatory chronic conditions. Nonetheless, its mechanism of action at the molecular level is not fully understood. In the liver, melittin - the main component of bee venom - is able to suppress the expression of pro-inflammatory cytokines through the nuclear factor kappa-beta (NF)- $\kappa\beta$  signaling pathway. Moreover, melittin reduces the activity of Hepatic Stellate Cells (HSCs) in vitro and decreases the expression of fibrotic gene responses in thioacetamide-induced liver fibrosis.<sup>81</sup>

Work by Kwon et al. has demonstrated that peripheral injection of bee venom significantly reduces or prevents the development of an inflammatory response in human arthritic disease.<sup>82</sup> Bee venom injection into the left hind limb reduces leukocyte migration in a zymosan-induced peripheral inflammation model.<sup>83</sup> Amin et al. suggest that some components of bee venom can cause inflammation by inducing interleukin 1 beta (IL-1 $\beta$ ) via a mitogen-activated protein kinase pathway: p38 MAPK, while others act as anti-inflammatory by suppressing inducible nitric oxide synthase (iNOS) and Cyclooxygenase-2 (COX2) via (NF)- $\kappa\beta$  in macrophages.<sup>84</sup> A more recent study by the same group using bee venom cross-linked to a hydrogel showed administration of this gel decreased inflammatory response and IL-6 production, and increased collagen formation in wounds in diabetic rabbits.<sup>85</sup>

## Oxidative Stress

Evidence suggests a link between oxidative stress and cellular damage and various diabetes associated complications. Loss of antioxidant defenses, together with a superfluous production of reactive oxygen species (ROS), play a crucial mediatory role in the pathogenesis and progression of chronic non healing diabetic wounds.

Honey contains antioxidants that scavenge free radicals.<sup>86</sup> The antioxidant potential of honey has been attributed to its phenolic content.<sup>87</sup> The high phenolic content of propolis has also been linked to its anti-oxidant properties. Studies by Sulaiman showed the free radical scavenging activities of propolis samples were strong when also evaluated by using the 2,2-diphenyl-1-picrylhydrazyl assay.<sup>88</sup> In a rat model of ischemic reperfusion injury, abundant amounts of ROS are produced, and CAPE, an active ingredient of honey bee propolis, was able to reduce tissue damage via its antioxidant effects.<sup>89</sup>

Enzymatic hydrolysates prepared from royal jelly showed anti-oxidative activity and scavenging activity against ROS.<sup>90</sup> In a separate series of studies, royal jelly time of harvest and the initial larval age had an effect on the antioxidant potencies in royal jelly. Royal jelly collected 24 hours after the larval transfer showed the most substantial antioxidant activities.<sup>91</sup> Few studies exist that explore the anti-oxidant potential of bee venom. In vitro studies by Rekka et al. showed that suppression of interleukin-1 production, offered by bee venom, may further support the theory that antioxidant activity is a mechanism of the anti-inflammatory activity of bee venom.<sup>92</sup> Bee venom was also shown to significantly decrease the level of ROS induced oxidative damage to synovial fluid proteins in a rat model of rheumatoid arthritis.<sup>93</sup>

## Bacterial Burden

Increased bacterial burden and wound infection are common in patients with diabetes and play an important role in ulcer chronicity. Honey has been shown to potently inhibit a range of bacterial pathogens, including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aerugi-*

*nosa*, and *methicillin-resistant staphylococcus aureus* (MRSA).<sup>94,95</sup> Honey has also been shown to work synergistically with systemic antibiotics and to have a low rate of adverse effects (occasional allergic reaction).<sup>96</sup> Honey promotes wound healing through regulating the moisture and preventing maceration balance in the wound bed via osmosis.<sup>97</sup> Its osmotic properties are also sufficient to inhibit microbial growth through dehydration of organisms.<sup>59,97</sup> Honey types that have a moderate level of antibacterial activity prevented the growth of *Staphylococcus aureus* if diluted beyond the point where their osmolality ceased to be inhibitory. Thus, the antibacterial activity cannot entirely be attributable to osmolality.<sup>98</sup>

Aside from its osmotic effect, honey exhibits multiple anti-bacterial effects. When honey is diluted by wound exudate, hydrogen peroxide is produced by the enzyme glucose oxidase.<sup>99</sup> Hydrogen peroxide is an effective antimicrobial agent when present at a sufficiently high concentration.<sup>100</sup> It also stimulates fibroblast proliferation in vitro and angiogenesis in vivo.<sup>101,102</sup> Phytochemicals, also known as flavonoids, are other antibacterial components of honey.<sup>103</sup> These compounds are found in high concentrations in commonly used therapeutic honeys (Manuka and Medihoney<sup>®</sup>) compared to other honeys. Various honeys have substantial non-peroxide antibacterial activity. Manuka honey, which is potently antibacterial, does not accumulate any hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>).<sup>104</sup> Manuka honey does, however, contain high levels of the antimicrobial compound methylglyoxal (MGO). The potential of this honey to inhibit the growth of *S. aureus* suggests that MGO is fully responsible for the non-peroxide antibacterial activity of Manuka honey.<sup>105</sup>

Robust evidence supports the anti-microbial properties of propolis.<sup>106</sup> Propolis has an inhibitory concentration that is 400 times greater than tetracycline's against *E. coli* and more than 50 times higher than tetracycline's against *S. aureus* and *B. subtilis*. Microcalorimetric analysis of propolis treated *M. Luteus* bacterial cultures was performed different growth phases. The addition of prepared propolis extracts to these cultures resulted in a strong decline in heat production,

a prolongation of the lag phase, and the introduction of a second lag phase indicating that the effects of propolis are both bacteriostatic and bacteriocidal.<sup>107</sup> The anti-bacterial activity of propolis is largely attributable to the phenolic acid fraction. Studies showed that no antibacterial activity against *S. aureus* was observed in a batch of propolis with a low phenol count compared to batches with a higher phenol count.<sup>108</sup> Propolis also inhibits the proliferation of fungal elements, such as *Candida* (at concentrations of 3-10mg/m)<sup>109</sup> and viruses.

Royal jelly shows inhibitory actions against Gram-positive bacterial strains *Bacillus subtilis*, *Micrococcus flavus*, and *Staphylococcus aureus* in a microplate assay, while showing no significant effect against gram negative bacteria.<sup>110,111</sup> Studies also showed that a royal jelly and starch mixture could be used to treat conventional drug resistant infections, and freshly reaped royal jelly was antimicrobial against *Pseudomonas aeruginosa*.<sup>112,113</sup>

Bee venom has antibacterial properties.<sup>114,115</sup> Studies by Han et al. that investigated the antibacterial activity of whole bee venom and purified melittin against *Escherichia coli* and *Staphylococcus aureus* in cow mastitis found it to be an effective antibacterial agent.<sup>116</sup> Lasioglossin-III is a new anti-microbial peptide (AMP) discovered in the venom of the *Lasioglossum laticeps* bee. It possesses broad spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria and has a low toxicity in solution.

### Immunomodulation

A relative lymphocyte response defect has been reported in chronic DFUs.<sup>117</sup> Consequently, the DFU healing process may be hampered by mechanisms which cause a decrease in the accumulation of leukocytes.<sup>117</sup> The immune-modulatory effects of honey include proliferation of peripheral blood B-lymphocytes and T-lymphocytes in cell culture at concentrations as low as 0.1% and phagocyte activation by honey at concentrations as low as 0.1%.<sup>118</sup> Propolis mediated inhibition of the p24 antigen by the human immunodeficiency (HIV) virus infected CD4(+) cells has also

been observed.<sup>119</sup> Royal jelly proteins MRJP1 and MRJP2 stimulate mouse macrophages to release TNF- $\alpha$ , whose primary function is to regulate immune cells,<sup>120</sup> whilst MRJP3 is able to modulate immune responses by suppressing the production of interleukins: IL-4, IL-2, and IFN- $\gamma$  in T cells.<sup>121</sup>

### Clinical Trials of 'Apitherapy' Products on Diabetic foot ulcers

Recently, many publications have attempted to quantify the efficacy of bee products as wound healing therapies using a variety of different wounds. Most clinical trials, in the context of 'Apitherapy', investigate the wound healing potential of honey. The healing deficiencies observed within DFUs have been long established. Promising pre-clinical data suggests that bee products may expedite these healing deficiencies, yet few clinical trials exist which investigate the effects of bee product therapies on DFUs. **Table 2** lists summaries and associated case studies.

Current evidence for using bee products on human DFUs is promising but weak. The studies are small, often anecdotal, and non-randomized. They also frequently lack controls. More robust trials are needed for such therapies to be accepted as an evidence-based treatment. Similarly, the Cochrane review of the randomized controlled trials (RCT) and quasi RCTs of honey used in chronic ulcers (including diabetic ulcers) was critical of the studies, citing broad inclusion criteria and failure to report on randomization as potential sources of bias.<sup>122</sup> Jull et al. concluded that:

'Honey dressings do not increase rates of healing significantly in venous leg ulcers when used as an adjuvant to compression. Honey may delay healing in partial- and full-thickness burns in comparison to early excision and grafting, and in cutaneous Leishmaniasis when used as an adjuvant with meglumine antimoniate. Honey might be superior to some conventional dressing materials, but there is considerable uncertainty about the reproducibility and applicability of this evidence.'

Table 2. Summary of clinical trials that have investigated the use of bee products to heal wounds. 'C' refers to the respective controls and 'H' to the type (if known) of honey used in the study.

Intervention	Author/Year/Reference	Experimental Design	Cohort	Therapy	Outcome
Honey	Moghazy et al. 2010128	Quasi experimental study	n=30 H=30	H= Honey	Decreased bacterial load after 1 week; 43.3% healed in 3 months; no controls
	Jan et al. 2008129	Quasi experimental study	n=100 H=50; C=50	H Honey C= Pyodine	Honey more effective than Pyodine dressings; increased healing rate; low amputation rate in honey group, but did not reach statistical significance
	Makhdoom et al. 2009130	Quasi experimental study	n=14 H=14	H= Honey soaked dressings	'Excellent results' reported; however, no timeframe or control group reported; 12/14 underwent amputation
	Eddy et al. 2008131	Case study	n=1	H= Honey smeared gauze	Ulcers that had been unhealed for 14 month; despite intensive treatment, healed in 6-12 months and remained healed after 2 years
	Kamaratos et al. 2010132	Randomized controlled trial	n=63 (n per group not stated)	H= Manuka honey dressings C=Conventional dressings	Honey treated ulcers healed in an average of 31 days; control wounds healed in an average of 43 days; P=0.05
	Shukrimi et al. 2008136	Randomized controlled trial	n=30 (n per group not stated)	H=Honey dressing C= Povidone iodine and saline	No significant differences in healing between groups; ulcers were healed adequately in 14.4 and 15.4 days respectively, to be ready for surgical closure
Propolis	Hossein et al. 2012133	Case study	n=1	P=Propolis + Olive oil	Ulcer of 2 months duration healed within 1 week
Royal Jelly	Sivash et al. 2011134	Quasi experimental study	n=8	R=Royal jelly	Mean healing time = 41 days; were fully 7/8 healed
	Sivash et al. 2013135	Randomized controlled trial	n=64 R=32; C=32	R=5% Royal jelly C= Placebo	No significant superiority to royal jelly treatment; RJ treated wounds healed in an average of 36 days compared to 38 days in controls; P=0.6

## CONCLUSION

Current pre-clinical research in the field of 'Apitherapy' products in wound healing suggests that honey, propolis, royal jelly, and bee venom are safe. They also have the potential to not only up-regulate healing in 'normal wounds' but also to attenuate the chronic inflammation, oxidative stress, bacterial burden, and immunodeficiency that thwarts healing in diabetic wounds.<sup>123, 124, 125</sup> The potential for bee products to be used as wound healing therapies has been established in terms of pre-clinical work, but more rigorous testing in the clinical setting is needed.

Within the hive ecosystem, honey and royal jelly are foodstuffs; bee venom is involved in defensive roles (killing intruders) and signaling (releasing pheromones that warn other bees of attack). The primary function of propolis is to protect bees against disease. Bees coat the internal walls of their hives with a thin layer of propolis to sterilize the comb and keep their hives bacteria free. Given this specific function, and that it has proven effective in pre-clinical studies, it is likely that among all bee products, propolis holds the greatest potential as a wound healing product.<sup>126</sup>

A lack of large-scale, well-conceived, robust clinical trials precludes bee products from becoming more accepted as wound therapies. Given the magnitude of diabetic foot ulcerations, there is an urgent need to systematically study bee products in human DFUs to determine if any may improve healing outcomes.

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