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PROPOLIS AND BEE VENOM IN DIABETIC WOUNDS; A POTENTIAL APPROACH THAT WARRANTS CLINICAL INVESTIGATION

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Abstract

Background: Wound healing in diabetes mellitus is a complex multi-stage process that requires the proper function of multiple systems. The mechanisms of impaired wound healing of diabetic wounds are still poorly understood. Therefore, various interventions are being used for wound management without great success. Bee products have various properties that make them an important addition to the diabetic wound management.
Methods: This review summarized previous and recently published papers of the effects of two bee products, propolis and bee venom, on the wound healing. The main results were obtained from preclinical experimentation.
Results: Diabetes mellitus compromises immune system, increases infections, impairs wound healing, and affects cells and factors involved in the wound healing. There is an increasing interest in natural products in modern medicine as part of disease management. Bee products are natural substances that others and we have explored some of their biological activities and applications in the treatment of various diseases. Some of these products are bee venom and propolis. These products have analgesic, antioxidant, antimicrobial, and anti-inflammatory properties. In addition, both propolis and bee venom contain considerable amounts of antioxidants that have a great role in accelerating wound healing.
Conclusion: There is sound rationality and scientific data for using propolis and bee venom in diabetic wound healing. We believe that topical application of propolis in addition to bee venom might have a place in repairing damaged tissues and accelerating the healing of diabetic wounds.

Key words: Honey, propolis, wound, cytokines, healing, diabetes, antioxidants, inflammation

Introduction

Diabetes mellitus (DM) is a systemic metabolic disease that is characterized by alterations in the metabolism of carbohydrates, proteins and lipids. DM compromises the immune system, increases the incidence of infection, and impairs wound healing. Many factors are involved in influencing the prevalence of DM; these include age, sex, genetic susceptibility, socioeconomic status, modern lifestyle and environmental factors (Zargar et al., 2000; Kadiki and Roaeid 2001).

In spite of modern interventions, complication of DM leads to blindness, amputation of limbs, end stage renal disease, and coronary artery disease. In diabetic wounds, DM compromises migration of leukocytes, impairs phagocytosis, increases oxidative stress (OS), causes advanced glycosylation of protein and enzymes, and exaggerates inflammatory response to microbial products (Soory, 2002**)** (Figure 1). In clinical practice**,** modern interventions such as daily wound care, hyperbaric oxygen, biological dressing, and surgery have limited success in the management of diabetic wounds. Furthermore, several types of wound dressing that are commercially available showed side effects and drawbacks **(**Young et al., 1991; Chakravarthy et al., 1994). New approach that includes nitric oxide (NO)-releasing materials is still under investigation **(**Shabani et al. 2001**;** Carpenter and Schoenfisch, 2012**).**

Consistent efforts to identify naturally occurring bioactive substances to treat human diseases have led to the discovery of potent natural products with marked bioactive properties. In this regard, bee products that include propolis, royal jelly, honey, beeswax, and bee pollen, are active biological substances that have a great potential to be used as an important part of diseases` management. These natural substances have been used for thousands of years by human cultures for the treatment of various diseases. Honey in particular has been mentioned in Holy books as a healer of diseases. It was mentioned in the Talmud, both the old and new testaments of the Bible, and in the Holy Quran. In the Surat Al-Nahel (The Bee) it says: And thy LORD taught the bee to build its cells in hills, on trees and in men's habitations, then to eat of all the produce (fruits) of the earth and find with skill the spacious paths of its LORD, there issues from within their bodies a drink of varying colours, wherein is healing for men, verily in this is a sign for those who give thought.

Both propolis and bee venom have antioxidant, anti-inflammatory, and antimicrobial activities that are important in accelerating wound healing. Data has demonstrated the effect of bee venom and propolis on wound healing. However, their effects on wound healing in diabetic ulcers have not been thoroughly investigated, and studies did not explore the underlying molecular mechanisms.

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Diabetic Ulcers

It is known that diabetic foot ulcers are the result of neuropathy and vascular complications of DM (**Table 1**). These complications usually end with limb amputation, sepsis and even death. It was found that delayed wound healing in DM reduces insulin sensitivity, causes the glycosylation of various proteins and enzymes by consistent hyperglycemia and the OS, and decreases antioxidant defense systems (Wolff, Jiang and Hunt 1991; Hallberg, Trocme and Ansari 1996; Bolajoko et al., 2008; Badr et al., 2012).

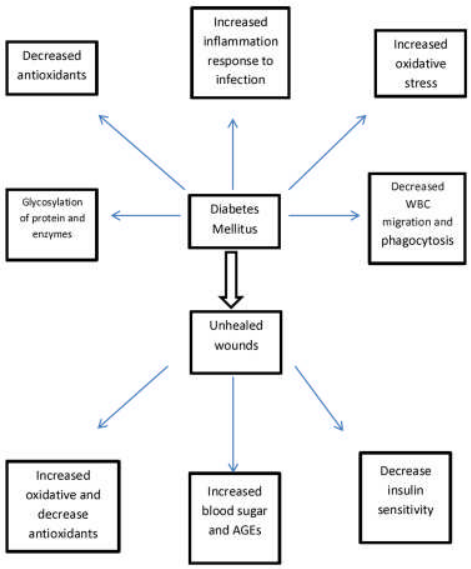

Figure 1: Effects of diabetes on wound

Abnormal proliferation of inflammatory cells in diabetic wounds is due to infection and inflammation-induced cytokines such as tumour necrosis factor-alpha (TNF-α) and interleukin (IL)-8 which suppress the proliferation of keratinocytes (Maas-Szabowski et al., 1999; Han et al., 2010).

Basically, wound healing in diabetic mice is characterized by a significant reduction in collagen deposition, decrease in the anti-inflammatory cytokine IL-10, reduction in the inflammatory phase, prolonged elevation in inflammatory cytokines (TNF-α, IL-1β and IL-6), activation of signal transducer and activator of transcription 3 (STAT3), reduction in the activation of protein kinase B and nuclear factor- κB (NF-κB), and decreased expression of β-defensin 2 and 3, vascular endothelial growth factor (VEGF), chemokines (macrophage inflammatory protein-1α, macrophage inflammatory protein -2, KC and CX3CL1) and of transforming growth factors (TGF-β) in wounded tissue (Badr et al., 2012). In addition, the elevated levels of IL-1β in conjunction with TNF-α decreases the expression of collagen mRNA via a pathway that increases matrix metalloproteinase (MMP) levels. Moreover, it was demonstrated that decreased expression of β-defensin 2 and 3 and increased incidence of bacterial infection are the main feature of delayed wound healing in animal`s model of diabetic wounds (Badr et al., 2012).

Recently, we found that diabetic mice exhibited delayed wound closure that was characterized by a significant decrease in the levels of TGF-β and prolonged elevation of the levels of inflammatory cytokines (IL-1β, IL-6 and TNF-α) and matrix metalloproteinase 9 (MMP9) in wound tissues. Moreover, the wound tissues of diabetic mice showed a marked reduction in the phosphorylation of Smad 2 and Smad 3 as well as a marked reduction in collagen production (Hozzein et al., 2015). Smads are intracellular proteins that transduce extracellular signals from transforming growth factor beta ligands to the nucleus. **Table 2** demonstrates the main features of diabetic wounds.

Regarding cellular element of diabetic wound, it was found that the wound-resident macrophages were abnormally short-lived and demonstrated an increased apoptosis with a significant reduction in their phagocytic activity (Badr et al., 2012). In this regard, our previous studies demonstrated that intravenous macrophages transfusion, both allogeneic and xenogeneic, accelerated wound healing and eradicated infections including septicemia in animals and in humans (Fakhri et al., 1984; Al-Waili, 1985; Al-Waili, 1989; Al-Waili and Al-Ani, 1989). Furthermore, intra-wound injections of macrophages have been shown effective to accelerate wound healing (Orenstein et al., 2005; Leor et al., 2006; Zuloff-Shani et al., 2010). Recently, the use of undenatured whey protein that has antioxidant activity in DM improved the healing and closure of diabetic wounds via the rescue of functional long-lived wound macrophages (Badr et al., 2012).

It is well known that unhealed wounds in DM are due to neuropathy, peripheral vascular disease, infection, local pressure, and local metabolic abnormality that were caused by hyperglycaemia. The effect of hyperglycaemia is summarized in **Figure 2**. Other main factors involved in delayed diabetic wound healing include decreased macrophage activity, persistence of neutrophils, defective chemotaxis, inhibited proliferation, excessive inflammation, fibronectin deficiency, accumulation of advanced glycation end products (AGEs), TNFα , MMP 1 and 9, Dipeptidyl Peptidase, and abnormalities in the growth factors (Schmidt et al., 1999; Liu et al., 2009 **;** Dasu et al., 2010; Landis et al., 2010). AGEs cause cellular ageing and structural modification of proteins; they bind to extracellular matrix (ECM) and modify its function, and also they induce cellular OS pathways (Schmidt et al., 1999). Prolonged inflammatory stage that is encountered in chronic wound is due to persistence neutrophil and accumulation of excessive amounts of MMPs, especially MMP-1 and MMP-9 (Chirife et al., 1983; Wetzler et al., 2000). Factors involved in

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persistent neutrophil in diabetic wound are decreased macrophage numbers, increased levels of apoptotic neutrophils, and increased pro-inflammatory mediators (Schleicher and Friess, 2007) (**Figure 3**). Another important factor that delays wound healing is bacterial burden that is increased in DM due to defective systemic and local immunity. Hyperglycemia increases superoxide generation which inactivates nitric oxide (NO) and reduces its bioavailability (Hink et al., 2001; Kim et al., 2002). NO is very important for wound healing. A review has summarized the beneficial effects of NO on wound repair and its role in angiogenesis, inflammation, cell proliferation, matrix deposition, and remodelling (Luo and Chen, 2005).

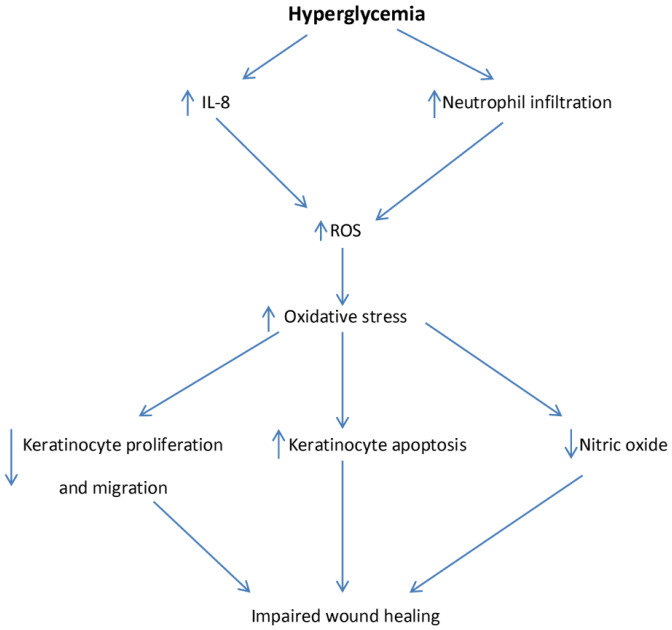

Figure 2: Consequences of hyperglycemia in wounds and ulcers

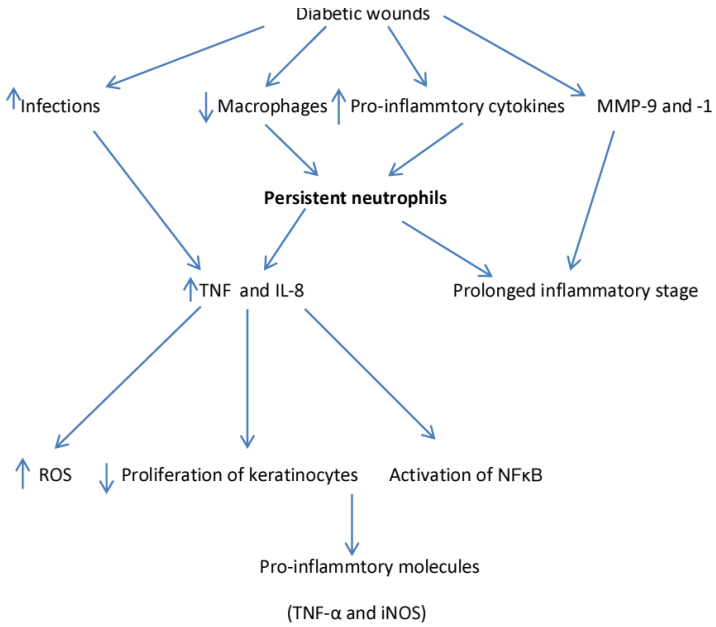

Figure 3: showed effects of persistent neutrophils in diabetic wounds

Table 1: Summary of the main causes of delayed wound healing in diabetes mellitus

Main causes of unhealed wound and ulcers in Diabetes mellitus
Neuropathy
Peripheral vascular disease
Microbial infection
Persistent pressure
Local metabolic abnormality caused by hyperglycemia.
Decrease macrophages activity,
Persistence of neutrophils,
Defective chemotaxis
Inhibited proliferation
Excessive inflammation
Fibronectin deficiency
Accumulation of advanced glycation end products (AGEs)- causes cellular ageing and it induces cellular OS pathways
↑TNFα
↑MMP 1 and 9
Dipeptidyl Peptidase
Abnormalities in growth factors

Oxidative Stress and Wound Healing

In wound healing, during the inflammatory stage, proteases and reactive oxygen species (ROS) are released and generated into the wound (Weiss, 1989). ROS play a role in cell signaling, immune response and OS during the process of wound healing. Data suggests that ROS such as hydrogen peroxide and superoxide are important regulators of wound healing (Schäfer and Werner, 2008). Inflammatory cells produce pro-inflammatory cytokines, IL-1β and TNF-α, which promote nuclear factor kappa-light-chain activation and ROS generation (Lowry, 1993).

Recent studies provided strong evidence for a role of OS in the pathogenesis of non-healing ulcers (Schäfer and Werner, 2008). Although Low levels of ROS are essential to stimulate wound healing, it has been found that elevated ROS increased IL-8 production and neutrophil infiltration in a high-glucose environment that contributed to impaired wound healing in diabetic skin (Lowry, 1993; Rodriguez et al., 2008; Guo and Dipietro, 2010; Lan et al., 2013). Another study showed that high level of ROS results in epithelial cell damage (Xu et al., 2009). In diabetic wound, high level of ROS causes excessive OS, decreased keratinocyte proliferation and migration and increased apoptosis (Nishio and Watanabe, 1997; Wenk et al., 1999; Deveci et al., 2005).

Proper wound healing requires a balance between OS and antioxidants. Basically, the normal physiology of wound healing depends on low levels of ROS and OS. High level of OS leads to impaired wound healing. Antioxidants enhance the healing of infected and non-infected wounds by reducing the damage that is caused by oxygen radicals (Martin, 1966). Many antioxidants are available over the counter or by prescription.

Table 2: Summary of the characteristic changes shown in diabetic wound (References; Chirife et al.,1983; Schmidt et al.,1999; Liu et al.,2009; Wetzler et al.,2000; Pierce 2001; Dasu et al., 2010; Landis et al.,2010;Badr et al.,2012; Schürmann et al.,2012; Hozzein et al., 2015)

Main changes encountered in diabetic wounds and ulcers
Decreased expression of B-defensin 2, 3
Increased incidence of infection
Decreased TNF-B
Increased MMB-9, IL-1B, IL-6, TNF, matrix metalloproteinase, IL-8
Decreased macrophages phagocytosis
Decreased Smad2 and Smad3 phosphoralation
Decreased expression of MIP-1 alpha, MIP-2, KC, CX3CL1
Decreased VEGF expression
Decreased IL-10
Decreased collage production

Bee Products and Wound Healing

Bee products have many biological activities and they are effective in various pathological conditions including wound healing. In this field, the main studies were conducted on honey that leads to introduction of honey as part of modern intervention in wound healing. Others and we have found that honey has antioxidant properties and various biological activities. In 1999, we have found that honey could accelerate post-surgical wound healing, shorten hospital stay and minimize scar formation (Al-Waili and Saloom, 1999). Honey has antimicrobial activities which were attributed to high osmolality, acidity, hydrogen peroxide generation, flavonoids, defensin and unidentified substances (Al-Waili et al., 2011; AL-Waili et al., 2013; Ansari et al., 2013; Al-Waili et al., 2014). Furthermore, we have found that honey reduces prostaglandins that can explain in part its anti-inflammatory activity (Al-Waili and Boni, 2003; Al-Waili, 2005). Other studies published from our laboratories showed that honey increased NO end products and this adds another explanation of its wound healing properties (Al-Waili, 2003; Al-Waili and Boni, 2004; Al-Waili et al., 2006).

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This paper summarizes the potential properties of other two bee products, bee venom and propolis, that make them potential future interventions in diabetic wound management.

Bee Venom

Bee venom therapy is well known in the complementary and alternative medicine. Bee venom therapy is thousands of years old and it involves the application of live bee stings to the skin or the injection of bee venom into the skin. Bee venom is produced by honey bees (Apis mellifera). It is obtained by electric stunning using a bee venom collector without harming the honey bees (Eskridge et al., 1981).

Bee venom contains at least 18 active components such as peptides, enzymes, and biogenic amines that showed pharmaceutical properties. It contains melittin, apamin, adolapin, phospholipase A2, mast cell degranulating peptide, α-D-glucosidase, biologically activity amines, hyaluonidase, acid phosphomonesterase, and lysophospholipase (Eskridge et al., 1981**;** Kwon et al., 2002) Melittin decreases cyclooxygenase (COX)-2 and phospholipase A2 expression and decreases levels of TNF-α, IL-1, IL-6, NO and ROS (Murakami et al., 1997;Amin, Attur, and Abramson, 1999; Cernanec et al., 2002). Melittin suppresses the expression of pro-inflammatory cytokines through the NF-κβ signalling pathway, and decreases the expression of fibrotic gene responses in thioacetamide-induced liver fibrosis (Park et al., 2007; Park et al., 2011).

Bee venom has anti-inflammatory, antibacterial, and anti-rheumatic activities. It has been used in arthritis, rheumatism, back pain, multiple sclerosis, cancer, and skin diseases (Franklin and Baer 1975; Eskridge **et** al., 1981; Somerfield et al., 1984; Liu et al., 2008; Cho et al., 2010; Jeong et al., 2014**)**. It relieves pain and inflammation and it has an immune response enhancing effect (Son et al., 2007). Bee venom is effective in burns and scarring (Han et al., 2007). Peripheral injection of bee venom significantly reduces or prevents the development of an inflammatory response in human arthritic disease (Kwon et al., 2002). In addition, bee venom can inhibit MMP-1 and MMP-3 production, which is useful for prevention of collagen damage (Fisher et al., 2002). Another study showed that bee venom reduces leukocyte migration in a zymosan-induced peripheral inflammation model (Kwon et al., 2003).

Anti-nociceptive and anti-inflammatory effects of bee venom have been demonstrated in rats and human by local subcutaneous injection of bee venom into an acupoint based on traditional Chinese medicine 'apipuncture' (Han et al., 2007). This procedure was used for various kinds of inflammatory pain models including complete Freund's adjuvant-induced arthritis model, formalin test, carrageenan-induced inflammation, intraperitoneal acetic acid writhing test, and neuropathic pain model (Chen et al., 1993; Kang, Pak and Choi, 2002**;** Lee et al., 2004; Kim et al., 2005). It was found that cytokines and NO, that are produced by bee venom, play an important role in mediating cell recruitment, activation of inflammation, and repair of damaged tissue (Petricevich, 2004).

The bee venom-loaded wound dressing composed of 10 % polyvinyl alcohol, 0.6 % chitosan and 4 % bee venom was examined in diabetic rats. The results demonstrated that the wound dressing accelerated healing of wounds and exhibited an anti-inflammatory effect. Furthermore, the wound tissues covered with this preparation displayed higher hydroxyproline and glutathione levels and lower IL-6 levels compared to the control (Amin and Abdel-Raheem 2014**).** In another study, the wound healing activities of chitosan and 6% bee venom - chitosan films were evaluated using excision, round section, full thickness skin wound model in rats (Amin, Abdel-Raheem and Madkor 2008). The results showed that 6% bee venom - chitosan films accelerated wound healing and inhibited PGE2; the anti-inflammatory effect was comparable to that of indomethacin. Studies showed that inflammation-induced cytokines such as TNF-α and IL-8 are secreted in wounds with infection and they acted to suppress the proliferation of keratinocytes (Maas-Szabowski et al., 1999; Han et al., 2010). In this respect, it was found that bee venom stimulated human epidermal keratinocyte proliferation and migration in vitro and also it decreased pro-inflammatory cytokines IL-8 and TNF-α expression levels in the keratinocytes (Sang et al., 2013). Recently, using bee venom cross-linked to a hydrogel decreased inflammatory response and IL-6 production, and increased collagen formation in wounds in diabetic rabbits (Amin, Abdel-Raheem and Madkor, 2008). Bee venom has also antibacterial properties (Fennell, Shipman and Cole, 1967; Fennell, Shipman and Cole, 1968, Amin, Abdel-Raheem and Madkor, 2008). It has activity against Escherichia coli and Staphylococcus aureus (Han et al., 2009). Other studies explore the anti-oxidant potential of bee venom (Rekka , Kourounakis and Kourounakis, 1990; Suh et al., 2006). It inhibited IL-1 and decreased level of ROS induced oxidative damage.

It was demonstrated that the impaired wound healing during DM is characterized by a reduction in the inflammatory phase in the first three days after injury (Badr et al., 2012), therefore, the use of bee venom which has inflammatory property and immune response enhancing effect might help wound healing by enhancing the inflammatory stimuli that are required for diabetic wound healing. Testing this hypothesis is currently in progress in our laboratories.

Obviously, bee venom has a potential action for wound healing by its anti-inflammatory, anti-microbial, analgesic and antioxidant properties (**Table** 3). In chronic unhealed wound, it might stimulate the inflammatory system and cytokines to activate the healing process.

Table 3: Effects of bee venom therapy on diabetic wound

Main effects of bee venom	**References**
Increases collagen formation	Amin et al.,2008
Has analgesic, anti-inflammatory, anti-oxidants and antimicrobial activity	Fennell et al., 1967; Fennell et al.,1968 Franklin & Baer 1975; Somerfield et al.,1984; Rekka et al., 1990; Suh et al.,2006; Liu et al.,2008; Son et al.,2007; Han et al.,2000; Cho et al., 2010; Jeong et al.,2014
Reduces leukocyte infiltration	Kwon et al.,2003
Inhibits MMP 1 and MMP 3	Jin et al.,2005
Inhibits prostaglandin E2	Amin & Abdel-Raheem 2014
Decreases IL-8, IL-6, TNF alpha and inflammatory responses	Han et al.,2013
Stimulates epidermal keratinocyte migration and proliferation	Han et al.,2013
Increases nitric oxide	Petricevich 2004
Decreases IL-6	Amin et al., 2008; Amin et al.,2014

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Propolis

Propolis is a natural plant product collected by Apis mellifera honeybees from various plant sources. Propolis is a resinous mixture of botanical balsams and resin with digestive enzymes of bees. The bees used propolis as the main colony-level defense mechanism against microorganisms and invaders and also it is used as a sealant in their hives. It contains more than 300 natural compounds including polyphenols, phenolic aldehydes, amino acids, steroids, sequiterpene-quinones, coumarins, and inorganic compounds (Farooqui and Farooqui, 2012).

Propolis has antimicrobial, antioxidant, immune-modulatory, anti-inflammatory, antitumor, antiulcer, hepato-protective, cardio-protective, and neuro-protective properties (Bilen, 2006; Sulaiman et al., 2011; Al-Waili et al.,2012; Omene et al.,2012; Talas et al.,2014). Most of its biological activity is attributed to flavonoids; terpenes; caffeic, ferulic and cumaric acids and esters. However, the chemical composition and biological properties of propolis depend on phytogeographical areas, seasonal collection time, and botanical sources (Farooqui and Farooqui, 2012).

Propolis has anti-inflammatory effects which are attributable to caffeic acid (Mirzoeva and Calder, 1996; Chan, Wen and Chiang 1995,; Ladwig et al., 2002). Caffeic acid phenyl ester is a potent inhibitor of MMP-9 that leads to decreased collagenolysis and pro-inflammatory proteinase which are increased in diabetic foot ulcers (Jin et al. 2005; Temiz et al., 2005; Liu et al., 2009). Furthermore, it was found that propolis treatment reduces the persistent inflammation that characterizes diabetic wounds by normalizing neutrophil and neutrophil elastase counts due to its anti-oxidant effects (Vieira et al., 1998; Mercan et al., 2006).

Propolis has bacteriostatic and bactericidal activities (Bankova et al., 1995; Gekker et al., 2005; Al-Waili et al., 2012; Astani et al., 2013). The anti-bacterial activity of propolis is largely due to the phenolic acid fraction (Prytzyk et al., 2003). Data showed that both acetone and ethanol extracts of two Bulgarian propolis samples have a comparable and a significant activity against Staphylococcus aureus and Candida albicans (Prytzyk et al., 2003). We have found that propolis has strong antimicrobial activity that was potentiated by the presence of honey (Al-Waili et al., 2012).

Propolis contains phenolic compound including flavonoids. They exhibit a wide range of biological activities (Fresco et al., 2006). They regulate cell signalling pathways, proliferation, migration, and survival, increase expression of anti-inflammatory genes, and inhibit MMP activities particularly MMP-9 **(**Majtan et al., 2010). Genistein, isoflavone in propolis, helped wound healing and improved wound angiogenesis in STZ-induced type-1 diabetes in mice by suppression of FoxO1, iNOS activity and OS (Tie et al., 2013). It normalized elevated oxygen radical's production and nitro-tyrosine formation, and reversed the low nitrite level that is seen in diabetes.

Recently, it was found that propolis increases generation of vascular NO (Talas, Gogebakan, and Orun, 2013). In liver tissue, propolis significantly increases NO levels when rats treated with Nω-Nitro-L-arginine methyl ester (Zeliha et al., 2015). Propolis from Brazil and Bulgaria was found to enhance the bactericidal activity of macrophages that involved the participation of hydrogen peroxide and nitrogen intermediate metabolites (Orsi et al., 2005). Another study showed that propolis increased hydrogen peroxide generation, anti-inflammatory cytokines and NO production by macrophages (Orsi et al., 2000). Recently it was found that the combination of NO donors and propolis can accelerate wound healing in experimental leishmaniasis (Miranda et al., 2015). Propolis has strong antioxidants that make NO available.

Studies have shown that propolis enhanced wound healing in different animal models including animals with diabetic wounds (Lotfy et al., 2006; McLennan et al., 2008; Kazancioglu et al., 2015). Furthermore, propolis may be effective in treating skin burns (Gregory et al., 2002; Ocakci et al., 2006). It was found that a single application of topical propolis helps ulcer closure, and reduces persistent neutrophil infiltration and elastase activity in the diabetic rodent model of full thickness cutaneous wounds (McLennan, et al., 2008).

Topically applied propolis was found effective to accelerate wound healing and to reduce the inflammatory response to silver nitrate-induced corneal alkali burns in rats (Martin et al., 2013). In burn inflicted on pigs, green and red propolis caused significant decrease of the inflammatory response, improved the epithelization rates, and induced earlier replacement of type-III for type-I collagen than collagen-based dressing films and control group (de Almeida et al.,2013). The myofibroblastic count and grosser interlacement of the collagen bundles were significantly increased by red propolis (de Almeida et al., 2013). Another study showed that in burn inflicted on pigs, propolis stimulates glycosaminoglycan accumulation that is needed for granulation, tissue growth, and wound closure, and it accelerates chondroitin/dermatan sulfates structure modification responsible for binding growth factors (Olczyk et al., 2013).

In a rat model, second-degree burns were inflicted in the neck region of female rats by contact with a hot metal for 5 seconds. The burns were treated either daily with 5% propolis ointment or by autologous amnion graft. The results revealed that propolis accelerates the process of tissue repair, leads to decreased local inflammation, and stimulates the production of collagen fiber (Pessolato et al., 2008). In the same animal model, topical application of propolis ointment for 14 days significantly improved wound contraction when compared to the control group of rats. The determination of hydroxyproline, hexosamine, uronic acid, DNA, RNA and protein levels in the wound matrix demonstrated the pro-healing effects of propolis (Iyyam Pillai et al., 2010).

In Diabetic rats, treatment of induced wounds with propolis prevented the decrease in epithelial closure and re-epithelialization. Propolis also prevented diabetes induced-impaired macrophage infiltration, persistent neutrophil infiltration, and increased myeloperoxidase activity (McLennan et al., 2008). Furthermore, it was found that oral green propolis (500 mg kg (-1)) administered post-implantation of polyether-polyurethane sponge discs in mice reduced inflammatory process in the sponge (de Moura et al., 2011).

Recently, we found that compared with untreated diabetic mice, topical application of propolis significantly enhances the closure of diabetic wounds and decreases the levels of IL-1β, IL-6, TNF-α and MMP-9 to near normal levels. Most importantly, compared with untreated diabetic mice, the treatment of diabetic mice with propolis significantly enhanced the production of collagen via the TGF-β/Smad2, 3 signalling axis in wounded tissues (Hozzein et al., 2015).

In humans, propolis is a useful topical treatment for ulcers (Wagh, 2013). In the clinical setting, 24 patients with diabetic foot ulcer were treated with topical propolis. The ulcer area was significantly reduced as compared to the control. In addition, the post-debridement wound fluid active MMP-9 and bacterial counts were significantly reduced (Henshaw et al., 2014). In patients with burn, propolis skin cream was found effective on the healing of partial thickness burn wounds (Gregory et al., 2002).

Aberrantly, topical applications of propolis might be beneficial in diabetic wound healing by its antibacterial and anti-inflammatory activities, and by its potent antioxidant constituents.

Table 4: the main beneficial effects of propolis on diabetic wound

Main functions of propolis on wounds and ulcers due to diabetes	References
Decreases MM-9, collagenolysis and proinflammatory proteinase	Jin et al.,2005; Temiz et al.,2008; Liuet et al.,2009
Has antimicrobial, immunomedulatory and antioxidant activity	Bankova et al.,1995; Gekker et al.,2005;Bilen 2006; Sulaiman et al.,2011; Al-Waili et al.,2012; Omene et al.,2012; Astani et al.,2013; Talas et al.,2014;
Reduces persistent neutrophils	Vieira et al.,1998; Mercan et al.,2006
Stimulates glycosaminoglycan	Olczyk at al.,2013
Accelerates chondroitin/dermatan sulfate modification	Olczyk at al.,2013
Stimulate collagen fiber via the TGF-β/Smad2,3 signaling axis	Pessolato et al.,2011
Increases macrophages infiltration	McLennan et al.,2008
Decreases IL-1 B, IL-6, and TNF alpha	Hozzein et al.,2015
Reduces elastase activity	McLennan et al.,2008
Increased myofibroblastic count	de Almeida et al.,2013

Conclusion

Data, mostly from preclinical studies, indicated that bee venom and propolis have potential therapeutic properties in chronic diabetic wound. If their therapeutic effects are proved by clinical studies, the use of propolis and /or bee venom will add new effective intervention that we are looking for in our clinical practice. These agents have the most desirable properties that are required in any successful intervention such as antimicrobial, antioxidants, anti-inflammatory and analgesic properties. Propolis and bee venom are natural, cheep, and almost lack of side effects. Future clinical studies should focus on advantage of using these agents over the current interventions, particularly in respect of therapeutic activities, safety, and cost effectiveness.

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