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Review

Honey Therapy in Diabetic Foot Ulcers: A Promising Strategy for Effective Wound Healing

Andrea Bezerra [1,2], Hélder Fonseca [2,3], Francisca Rodrigues [4], Cristina Delerue-Matos [4], Irene Gouvinhas [1,*,†] and Juliana Garcia [1,5,*,†]

1 CITAB—Centre for the Research and Technology of Agro-Environment and Biological Sciences, Inov4Agro-Institute for Innovation, Capacity Building and Sustainability of Agri-Food Production, University of Trás-os-Montes e Alto Douro, 5001-801 Vila Real, Portugal; dea.beatriz@hotmail.com
2 Research Center in Physical Activity, Health and Leisure (CIAFEL), Faculty of Sport, University of Porto, 4200-450 Porto, Portugal; hfonseca@fade.up.pt
3 Laboratory for Integrative and Translational Research in Population Health (ITR), University of Porto, 4200-450 Porto, Portugal
4 REQUIMTE/LAQV, ISEP, Polytechnic of Porto, Rua Dr. António Bernardino de Almeida, 431, 4249-015 Porto, Portugal; francisca.rodrigues@graq.isep.ipp.pt (F.R.); cmm@isep.ipp.pt (C.D.-M.)
5 AquaValor—Centro de Valorização e Transferência de Tecnologia da Água–Associação, Rua Dr. Júlio Martins n.º 1, 5400-342 Chaves, Portugal
* Correspondence: igouvinhas@utad.pt (I.G.); juliana.garcia@aquavalor.pt (J.G.)
† These authors contributed equally to this work.

Abstract: Diabetic foot ulcers (DFUs) are considered a major problem for public health, leading to high rates of lower-limb amputations. Moreover, due to the high prevalence rate of predisposing factors, the incidence rate of DFU is still rising. Although DFUs are complex in nature, foot ulceration usually precedes diabetic foot amputations. These impaired chronic wounds usually promote a microbial biofilm, commonly characterized by the presence of multidrug-resistant microorganisms, hampering the efficacy of conventional antibiotic treatments. Honey has been shown to be an effective antibacterial component, including against multidrug-resistant bacteria. Honey's physical–chemical characteristics, such as the presence of hydrogen peroxide, its low pH levels, and its high sugar and phenolic contents, promote anti-inflammatory and antioxidative activities, improving wound healing. This review aims to explore honey's effects in wound healing, especially for DFUs, and to show how the different physical–chemical features among different honey types might influence the treatment's effectiveness. For this, the mechanisms by which honey can promote wound healing and the potential use of honey dressings in diabetic wounds were investigated in animal models and humans. After revising the diabetic wound impairment mechanisms, we found that most of the clinical studies that treated DFUs with honey in animal models or humans reported accelerated wound healing, greater wound contraction, and lower amputation or hospitalization rates; however, few studies characterized the features of honeys used for wound treatment, hindering the possibility of extensively comparing the different types of honey and identifying characteristics that most successfully promote wound healing. According to this review, honey is a cost-effective and safe option for DFU management.

Keywords: honey; diabetic foot ulcer; diabetic wounds; wound dressing

Citation: Bezerra, A.; Fonseca, H.; Rodrigues, F.; Delerue-Matos, C.; Gouvinhas, I.; Garcia, J. Honey Therapy in Diabetic Foot Ulcers: A Promising Strategy for Effective Wound Healing. *Appl. Sci.* **2023**, *13*, 12820. https://doi.org/10.3390/app132312820

Academic Editors: Malgorzata Dzugan and Dorota Grabek-Lejko

Received: 18 October 2023
Revised: 23 November 2023
Accepted: 24 November 2023
Published: 29 November 2023

1. Introduction

According to the International Diabetes Federation (IDF), in 2021, 537 million adults had diabetes, and this number is expected to reach 783 million by 2045 [1]. Among the worst complications of diabetes, the development of diabetic foot ulcers (DFUs) is one of the major public health challenges [2]. Almost 20% of patients with diabetes might develop DFUs [2], with a prevalence rate of amputations of 24.5% [3]. In fact, DFUs

are considered the major cause of lower-limb amputations [4]. The hyperglycemic and inflammatory state promoted by diabetes can impair wound healing through multifactorial mechanisms [5,6], leading to the development of chronic wounds and ulcers, and in the latter case to limb amputation [7]. Furthermore, diabetic wounds (DWs) are usually colonized by a microbial biofilm with multidrug-resistant microorganisms, reducing the efficacy of antibiotic treatments [8]. Despite many advances in bioengineering to promote the best DFU management approaches [9], concerns regarding the bacterial resistance usually found in DWs [10–12] and the high costs associated with DFU treatments are still major hindrances to promoting the best wound dressing options for patients [13,14].

In order to promote a cost-effective treatment and avoid drug side effects, natural sources have been extensively studied for DFU management [13,15,16]. Among them, a growing body of evidence shows that honey has antioxidant, anti-inflammatory, and antibacterial activities, including against multidrug-resistant bacteria [17]. Furthermore, honey's characteristics promote a physical barrier against external microbes, as well as a suitable acidic and moisturized environment that favors wound healing, including in DWs, through different mechanisms [18]. All of these benefits make honey a potential DFU treatment [19]. In this context, this review aims to review the mechanisms of impaired wound healing in diabetes and honey's antimicrobial and anti-inflammatory activities and to explore DFU models treated with different types of honey dressings.

2. Diabetes

Diabetes mellitus (DM) is a clinical syndrome characterized by high blood glucose levels, mainly related to a deficiency or absence of insulin secretion by β-pancreatic cells or a deficiency in peripheral insulin signaling [20]. In 2030, the number of people living with diabetes is expected to increase by 25% based on the reported number of cases in 2019 [21]. Patients with diabetes are likely to suffer from many complications related to this condition, such as nephropathy, retinopathy, and neuropathy [22]. Among the different types of diabetes, type 1 and type 2 are the most prevalent [23,24], with type 2 comprising almost 90% of reported diabetic cases [25]. Whereas type 1 diabetes mellitus (T1DM) is related to an autoimmune response, leading to β-cell destruction and insufficient insulin production, type 2 (T2DM) is mainly characterized, at least in the early phase of the disease, by insulin resistance [25]. Patients with T1DM are more likely to develop microvascular complications, while T2DM patients are more prone to macrovascular complications [24]. The development of diabetic foot is considered a major public health problem, since the patients present with impaired wound healing, leading to diabetic foot ulcer (DFU) development [26].

2.1. Diabetic Foot Ulcers

Diabetic wounds (DWs) represent one of the most frequent and devastating complications of DM, directly impacting patients' morbidity and quality of life [5]. DWs might differentiate according to internal or external origins. The wounds of external origin, such as from cuts and injuries, might remain unnoticed by patients due to the peripherical neuropathy caused by diabetes [6]. On the other hand, the wounds of internal origin, such as ulcers and calluses, cause skin destruction and have an increased rate of bacterial infection [6]. In such cases, the DFU begins as a superficial ulcer, progressing to a deep-tissue infection and then in the final step to osteomyelitis [27]. The three major factors contributing to DFU are neuropathy, vasculopathy, and infection [28].

2.1.1. Diabetic Peripheral Neuropathy and Vasculopathy

Diabetic neuropathy is a neurodegenerative disorder of the peripheral nervous system that targets sensory, autonomic, and to a lesser extension motor neurons [29]. Foot ulceration can develop via motor neuropathy due to the foot's intrinsic muscle weakness; sensory neuropathy, which promotes unnoticed trauma; and finally autonomic neuropathy, which decreases sweating and leads to xerosis development [9]. Peripheral nerve dysfunction has

been strongly correlated with microvascular complications caused by diabetes [23]. In fact, the prolonged inflammation status caused by hyperglycemia in the microcirculation might lead to the thickening of the capillaries' basement membrane and endothelial hyperplasia, which impair nutrient and white blood cells movement, leading to tissue ischemia [23]. Microvascular disease can also affect the nervous system through nerve fiber deterioration, altering the thermal and vibration sensitivity thresholds, thereby favoring the development of neuropathy [20]. In this sense, small undetected wounds may turn into serious complications, such as DFUs.

Diabetes arterial hypertension is also another suggested risk factor for microvascular dysfunction, neuropathy, and cardiovascular disease development [23,30]. The evidence suggests that controlling hypertension levels in patients with diabetes through the use of medicaments decreased the risk of developing microvascular and large-vessel dysfunctions [31]. In diabetes, the endothelial dysfunction and inflammation caused by large-vessel diseases, such as atherosclerosis and vascular calcification, are amplified, increasing the risk of DFU development [30]. Despite both micro- and macrovascular diseases being considered risk factors for peripheral arterial disease (PAD), a five-year follow-up with T2DM patients showed that only microvascular disease was an independent predictor of PAD, being associated with lower-limb ulceration development and amputations [32].

2.1.2. Infection and Wound Healing

In general, wounds can be characterized as acute, which have a normal healing process, and chronic, where the healing process is impaired [33]. A normal wound healing process is composed of four overlapping steps, namely hemostasis, inflammation, proliferation, and remodeling [34]. These phases are orchestrated by different cell types that progressively operate within the wound milieu, namely monocytes, macrophages, neutrophils, keratinocytes, and fibroblasts [35,36]. The first phase is characterized by the release of growth factors by the platelet plug that is formed during primary hemostasis, such as transforming growth factor beta (TGF-β) and epidermal growth factors [5]. During the subsequent inflammatory phase, neutrophils recruited to the wound site initiate phagocytosis to remove foreign materials, bacteria, and damaged tissue [34]. Monocytes are stimulated to migrate to the tissue and release inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin 6 (IL-6) [37]. After neutrophil infiltration ceases, macrophages continue the phagocytosis process to clean the wound site in preparation for the next phase [33,34]. Following the proliferative phase, the processes of angiogenesis, collagen deposition, granulation tissue formation, re-epithelialization, and wound contraction occur [18]. The re-epithelialization process results from the keratinocytes' migration and proliferation to the skin surface. Afterwards, during the remodeling phase, the previously deposited collagen is remodeled and the unnecessary cells present in the wound undergo apoptosis [33].

The chronic wound state is characterized by a persistent injury state during wound healing due to the lack of or the severe slowdown of at least one of the healing phases [35]. Chronic wounds are usually infected and exhibit a persistent aberrant inflammatory profile, with hyperproliferative keratinocytes, elevated matrix metalloproteases, and poor fibroblast infiltration and angiogenesis [36,38]. Chronic wounds represent one of the major public health issues worldwide, impairing patients' quality of life and mobility [39]. Almost 90% of chronic wounds contain microorganisms such as bacteria and fungi, creating a multispecies biofilm that protects them from antimicrobial therapies and the immune system [6,8]. Diabetic foot infections caused by *Pseudomonas aeruginosa*, *Escherichia coli*, *Citrobacter* spp., *Acinetobacter* spp., and *Staphylococcus aureus* usually develop non-healing chronic wounds [16]. The polymicrobial biofilm infection can impair wound healing with more than a single bacterial infection, becoming even more resistant to antimicrobial therapies [40].

The wound healing process in patients with diabetes is impaired due to the multifactorial disruption of cellular coordination and the molecular process underlying wound

repair [5]. Moreover, diabetes causes microvascular complications, favoring the local wound ischemia and delaying the healing process [6]. The increased blood viscosity, dysfunctional activity of polymorphonuclear neutrophils, and declined delivery of nutrients and oxygen to the wound site promoted by diabetes are also important factors that justify the wound healing delay [33]. Moreover, DWs usually exhibit a persistent inflammatory phase, impairing tissue granulation and the connective tissue's tensile strength [6].

Diabetes impairs the functions of neutrophils and macrophages, including cell adherence, chemotaxis, phagocytosis, and cytokine production and secretion [41]. The hyperglycemia and oxidative stress that diabetes patients are exposed to lead to epigenetic changes, thereby altering the macrophages' polarization [42]. In fact, lower numbers of macrophages are found in DWs, contributing to impaired tissue repair [43]. Macrophage migration inhibitory factor (MIF) was also found to decrease in DM [44], which could affect the recruitment of endothelial progenitor cells [45].

Beyond macrophage dysfunction [43], animal models of diabetes show impaired fibroblast response to growth factor stimulation [46], as well as impaired keratinocyte and fibroblast migration and proliferation [47]. Diabetes might also impair wound healing by decreasing the growth factors responsible for the extracellular matrix formation, such as keratinocyte growth factor (KGF) and fibroblast growth factor (FGF) [48,49]. Beyond this, the expression of receptors can also be reduced, such as transforming growth factor β receptor type 1 (TGF-βR1), which is responsible for fibroblast-to-myofibroblast differentiation during the granulation tissue formation process, leading to deficient re-epithelization and wound contraction [50]. In fact, IGF-1 expression in animals with diabetes was also reported to be delayed and decreased during the healing process when compared with non-diabetic animal models [51].

Delayed vascular regeneration was also observed in diabetes due to many factors, such as receptor dysfunction, a reduction in the number of essential ligands or cells, and impaired epithelial-to-mesenchymal transition (EMT) [49]. In fact, a reduced functional capillary density and decreased angiogenesis-positive area were observed in animal models with diabetes, showing impaired vascular regeneration [26]. Moreover, the levels of the transcription factor zinc finger E-box binding homeobox 1 (ZEB1) were observed to be responsive to the hyperglycemia status, and the dysregulation in ZEB1 expression led to a defective EMT towards wound epithelialization and poor angiogenesis [52].

The severity of DFU is classified by the Wagner system into grade I, indicating a superficial uninfected ulcer; grade II, indicating a deep ulcer with exposed tendons; grade III, indicating a deeper ulcer with exposed bone and infection; grade IV, indicating partial gangrene of the foot; and grade V, indicating complete gangrene [7]. In a study performed in 194 patients with DFU grades between I and III, 16% did not heal in 6 months, 15% underwent amputation, and 4% died [53]. Therefore, diabetes is known to compromise wound healing via multifactorial mechanisms, increasing several concerns about the development and prevalence of DFUs in this population.

2.1.3. Types of Treatment for Wound Healing and their Limitations

The standard treatment of DFUs comprises wound debridement, offloading, and infection control with antibiotics [3]. This involves removing infected and necrotic tissue with a scalpel, providing a specialized cast model to distribute the body's weight in the foot, and controlling infection through the use of antibiotic treatments, respectively [3]. In this sense, wound management follows the TIME concept (tissue control, infection–inflammation, moisture balance, and edge of wound) [27]. Tissue control corresponds to wound debridement, infection–inflammation comprises the control of the bioburden continuum, moisture balance is related to regulating the wound exudate and restoring the moisture balance, and finally the edge of the wound comprises the promotion of epithelial advancement [27].

The ideal wound dressing material must be non-adherent to the wound, be able to create and maintain a moist environment, reduce the excess of wound exudate, and allow

gaseous exchanges [54]. Since a reduction in wound healing time is crucial in lowering the risk of infection and complications in DWs [16], new alternatives such as bioengineered tissue products and natural and synthetic skin grafts have been developed for wound healing [9,55]. In this sense, biomaterials, such as alginates, collagen, fibronectin, and chitosan, have been exploited to accelerate wound healing, stimulating cell proliferation and angiogenesis [55] in the form of nanofibrous bandages, films, hydrogels, hydrocolloids, tulle, foams, or gauzes [20]. For instance, a chitosan topical gel and film were effective in promoting tissue granulation and DFU closure [56].

The development of a three-dimensional scaffold improves the wound healing result even more, since it acts as a wound dressing, protecting against external infections and also providing an appropriate surface chemistry with nano- and microstructures that facilitate cellular attachment, proliferation, and differentiation [14]. Furthermore, scaffolds are biodegradable, and upon application at the wound site they start to degrade themselves and release drugs in a time-dependent manner into the wound [9]. Despite all of these alternatives, many of these treatments are still expensive, require extensive care, and do not full recover the skin's functionalities [13,14].

For infection control, the treatment with antibiotics might be performed based on a previous culture of the infected tissue to assess the bacterial colonization profile. The most reported antibiotic therapy options for DFUs in clinical trials are cephalexin and amoxicillin–clavulanate for mild infections and ampicillin–sulbactam, ertapenem, imipenem–cilastatin, vancomycin, and piperacillin–tazobactam for moderate to severe infections [57]. The systemic administration of antibiotics has been considered an increasing limitation, especially against multidrug-resistant bacteria [16,58]. Standard antibiotics seem to have a minimal long-term effect on treating chronic wounds, since they are not able to fully penetrate biofilms or attack all species of bacteria embedded in the extracellular polymeric matrix [59]. In the European Union, antibiotic resistance causes 25,000 deaths per year [60], and if not controlled this number might reach 10 million deaths worldwide by 2050 [61].

Among the multidrug-resistant pathogens, *S. aureus*, methicillin-resistant *S. aureus* (*MRSA*), *P. aeruginosa*, *E. coli*, extended-spectrum β-lactamase-producing (ESBL) *E. coli*, and vancomycin-resistant *enterococci* (*VRE*) have been reported as being challenging in infection treatment [12]. Importantly, 18% of the hospitalized patients with DFUs were positive to multidrug-resistant organisms, mostly *MRSA* [10]. Similarly, another study reported the presence of resistant bacteria in 21.8% of patients, of which 62.7% also presented with *MRSA* [11]. Despite efforts toward the development of new antibiotics, the extent of bacterial resistance is increasing worldwide and represents a great concern for public health [62]. In this context, alternative treatments have been increasingly stimulated to overcome the growing bacterial resistance to available conventional therapy [15].

In order to promote more accessible and efficient treatments with reduced side effects and risks, natural therapies have been increasingly investigated to treat microbial infections in DFUs. For instance, herbal products, such as curcumin [13], allicin [63], and *Aloe vera* [64], possess antimicrobial, anti-inflammatory, and antioxidant activities that accelerate wound healing [16]. Similarly, traditional Chinese medicines such as Tangzu Yuyang ointment [65] and *Centella asiatica* [66] have also been considered options in the management and healing of DFUs due to their anti-inflammatory effects. Although these options hold potential benefits for wound management, the antibacterial effects of traditional Chinese medicines are still not very well documented in vivo for DFU treatment [27].

Honey is among the natural resources with major potential to promote wound healing, since it is accessible and has convincing antibacterial, antioxidant, and anti-inflammatory properties [17]. Furthermore, honey's biological structure meets several of the ideal wound dressing requirements, since it provides a moist environment for wounds and protects against injury during dressing changes [18,67]. All of these advantages make honey a promising and cost-effective natural medicine to treat DFUs [19].

3. Honey as an Antimicrobial and Anti-Inflammatory Agent

Honey is considered one of the older wound dressing biomaterials [18]. Gifted with anti-inflammatory, antioxidant, and antibacterial characteristics, honey is a natural substance that is frequently reported as a traditional medicine and has been increasingly investigated [17], especially for chronic wound treatment [68]. The current evidence shows that honey's composition and antimicrobial properties depend on the geographic conditions, the surrounding environment of the hive, and the metabolic activity of the bees, as well as the processing and storage conditions, which will confer distinct characteristics and levels of effectiveness on different microbial strains [18,69]. For instance, manuka honey, a honey derived from the nectar of the *Lepstospermum scoparium* (manuka) plant in New Zealand, is recognized for its high concentrations of methylglyoxal (MGO), which confer to pronounced antibacterial activity [70]. The presence of polyphenols is another important factor that contributes to a honey's color, taste, and antioxidant and antimicrobial characteristics, which depend on its floral sources [71]. Despite the differences related to a honey's geographic and botanical origins, almost all types of honey present bactericidal activity as an intrinsic promising property for drug development [72].

Honey is effective against more than 60 bacterial species [73] and has been widely used in the treatment of acute and chronic wounds, ulcers, and burns [74]. Interestingly, honey is also able to inhibit bacterial cell wall synthesis, changing the cell shape and inducing lipopolysaccharide outer membrane disintegration [72]. In vitro studies carried out on wound healing have shown the benefits of honey, not only as a general antibacterial medicine [39,75] but also in the treatment of multidrug-resistant bacteria [76,77]. Different types of honey, such as buckwheat (*Fagopyrum esculentum*), blueberry (*Vaccinium corymbosum*), and manuka, have shown great effectiveness against *E. coli* and *Bacillus subtilis*, as well as vancomycin-resistant *Enterococcus faecium* and *MRSA* [78]. The effectiveness of honey against multidrug-resistant bacteria was shown in a recent review [12] and it has gained increasing attention, especially due to the worldwide antibiotic resistance concerns. In this sense, honey was reported as an effective treatment for chronic wound infections not responding to antibiotics [79].

During the wound healing process, honey has a modulatory role in the inflammatory phase, avoiding a chronic or severe inflammatory state being reached [18]. Importantly, there is some evidence that honey inhibits the nuclear factor kappa B (NF-kB) pathway through NF-kB nuclear translocation attenuation, decreasing the inflammatory mediators, such as cyclooxygenase-2 (COX-2) and TNF-α [80]. Conversely, the activation of macrophages and the stimulation of COX-2, TNF-α and prostaglandins (PGE2) were also observed after treatment with thyme honey [35], suggesting that honey is a immunomodulator of wound healing. This means that honey provides pro-inflammatory and anti-inflammatory properties that are activated according to the wound characteristics [81]. A reduction in the reactive oxygen species (ROS) released from infiltrated neutrophiles is another major mechanism by which honey can decrease the cells' oxidative stress levels and reduce the inflammatory status [81,82]. Japanese honey also showed effectiveness in reducing the wound area during the inflammatory phase [34]. During the proliferative phase, honey is also able to stimulate angiogenesis [83] and wound contraction through fibroblast, myofibroblast, and collagen deposition [18], despite improve the re-epithelialization [84], possibly due to the high osmotic pressure and presence of hydrogen peroxide [83]. During the remodeling phase, honey also shows benefits in decreasing scarring and improving tissue remodeling [85] (Figure 1).

The in vitro evidence suggests that honey might initiate healing in recalcitrant wounds by accelerating several mechanisms such as the desquamation of devitalized tissue, the mobilization of macrophages to the wound bed, increasing cellular energy sources, enhancing granulation and epithelization, and preventing infection [83]. Moreover, honey might improve wound healing by preventing the biofilm formation of *S. aureus*, *Streptococcus pyogenes*, and *P. aeruginosa* by impairing bacterial binding at wound sites and on the surfaces of keratinocytes [86]. In mature wound biofilms, manuka and honeydew honey were also

able to decrease the viability of *S. aureus*, *Streptococcus agalactiae*, and *P. aeruginosa* within 48 h [39]. This evidence suggests an advantage for honey in chronic wound treatment.

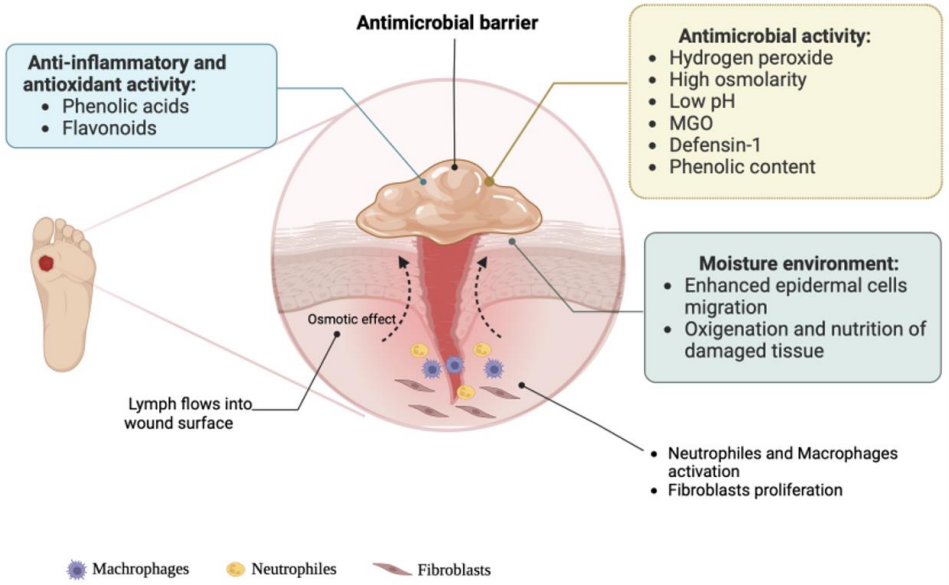

Figure 1. Honey's potential mechanisms of wound healing.

In vivo, honey is widely reported as a safe, cost-effective, and beneficial dressing biomaterial for wound healing [18]. C57BL/6J mice treated with 50 µL of undiluted indigenous New Zealand rewarewa honey were able to inhibit edema and leukocyte infiltration after four hours of application, supporting honey's anti-inflammatory activity, which targets the neutrophil respiratory burst, recruitment, and swelling [87]. In humans, treating acute wounds such as burns with honey significantly reduced the healing and infection time due to honey's antimicrobial, cell proliferation, and anti-inflammatory effects [88]. Similarly, chronic wounds, such as venous leg ulcers, treated with honey demonstrated increased rates of healing with lower rates of infection than with hydrogel [89]. In patients with pressure ulcers at stages II and III, five weeks of treatment with gauze dressings embedded with monofloral unprocessed honey decreased the ulcer size when compared to patients treated with ethoxy-diamino-acridine and nitrofurazone dressings [90], showing honey's effectiveness in wound healing as an advanced treatment.

4. Honey's Antimicrobial Properties

4.1. Hydrogen Peroxide as the "Inhibine"

Hydrogen peroxide (H_2O_2) is an oxidizing and biocidal agent [91]. The oxidation process can generate hydroxyl radicals, culminating in microbial inhibition and DNA damage [78]. The current evidences assume that H_2O_2 is responsible for the major antimicrobial action of honey [92]. The detection of honey's antimicrobial action was first described by Dold et al. [93], who described the antibiotic agent as "inhibine". Afterward, similarities between honey's antimicrobial properties and the responses of H_2O_2 to heat and light suggested that H_2O_2 was the major antimicrobial agent in honey [94]. Later, an experimental analysis showed that the H_2O_2 concentrations were directly proportional to the enzymatic activity and to the "inhibine number", showing H_2O_2 as the "inhibine" previously documented [95]. Finally, the decomposition of H_2O_2 by catalase in honey samples led to a reduction in antimicrobial action of the honey [94]. This growing evidence supports the updated postulation that H_2O_2 is one of the most important antimicrobial agents in honey.

H_2O_2 is produced enzymatically due to the presence of glucose oxidase, which is released by bees into nectar during the production of honey [92]. Thus, the levels of H_2O_2 depend on the amount of glucose oxidase added to honey samples and the presence of

pollen-derived catalase [96]. The level of dilution also affects the H_2O_2 concentration [97], as can be observed by the lower H_2O_2 levels found in undiluted honey, possibly due to the glucose oxidase inactivation in the presence of full-strength honey's lower pH [95]. Higher levels of glucose oxidase can be observed in diluted honey, which lead to greater H_2O_2 concentrations [98], increasing honey's bactericidal potential. Endogenous concentrations of H_2O_2 lead to concentration-dependent DNA degradation [96]. Moreover, honey samples with low contents of H_2O_2 that were unable to degrade DNA recovered this potential when supplemented with exogenous H_2O_2 [96]. A strong correlation was also observed in buckwheat, blueberry, and sweet clover honey samples between the bacterial activity against *E. coli* and H_2O_2 content [99]. Conversely, the removal of H_2O_2 from honey can abolish the bacterial DNA degradation and can even have a protective effect on bacterial DNA [96]. Similarly, honey activity against *E. coli* and *P. aeruginosa* was strongly reduced after H_2O_2 neutralization [79]. Curiously, the removal of H_2O_2 might affect each type of honey differently, altering in a singular way their properties. For instance, the introduction of catalase to buckwheat honey decreased the antibacterial effects against *E. coli*, whereas in manuka and blueberry honeys the treatment results were not affected [78].

The antimicrobial activity of H_2O_2 is usually related to oxidative stress originating from hydroxyl free radicals reacting with lipids, proteins, and nucleic acids [100]. It has also been suggested that H_2O_2 exerts an antimicrobial activity through the active hydroxyl free radicals released by the catalytic action of the traces of metal ions from bacterial strains [101,102]. Importantly, cells with elevated intracellular iron concentrations showed greater susceptibility to H_2O_2's activity [103].

A bimodal mechanism of action is usually reported according to the H_2O_2 concentration [104]. Some evidence suggests that at low H_2O_2 concentrations (<2.5 mM), the first-mode antimicrobial activity against *E. coli* occurs due to bacterial DNA degradation, whereas in the second mode, at high H_2O_2 concentrations (10–12.5 mM), hydroxyls radicals are the major factor responsible for killing bacteria [104,105], with the exposition time, rather than the H_2O_2 concentration, being the major bactericidal factor [104]. Distinct bacterial strains (*Ralstonia metallidurans*, *E. coli*, *Shewanella oneidensis*, and *Deinococcus radiodurans*) submitted to different concentrations of H_2O_2-induced oxidative stress were monitored using flow cytometry, revealing that the membrane potential, esterase activity, intracellular pH, and superoxide anion production were affected when exposed to high H_2O_2 concentrations [106]. Moreover, the different effects observed in the cellular membrane in response to oxidative stress suggest that the different membrane properties among bacterial strains might be important factors used to determine the bacterial stress resistance to H_2O_2 [106]. Therefore, the extending damage promoted by H_2O_2 might depend not only on the H_2O_2 concentration but also on the bacterial sensitivity to oxidative stress.

4.2. Non-Peroxide Antimicrobial Activity

4.2.1. Acidity and pH

Honey usually presents an acidic environment with pH values ranging between 3.2 and 4.5 [97,107] due to the formation of gluconic acid from glucose catalysis by glucose oxidase [108] As with its other characteristics, honey's acidity also depends on its botanical origin [15]. Low-pH environments have demonstrated effectiveness in inhibiting the activity of microorganisms, and in honey this seems to be one of the most important characteristics for chronic wound healing [109], since such wound promote a neutral to slightly alkaline environment that is more favorable to microbial infection [18,110]. In fact, the optimal pH range for microorganism growth is from 6.5 to 7.5, meaning honey's acidity can drastically inhibit the microbial proliferation [74]. The evidence suggests that after neutralizing H_2O_2, MGO, and defensin 1 (Def-1), honey's low pH was the responsible factor acting against *B. subtilis* [79]. Honey's acidic environment is also related to increases in oxygen release from hemoglobin in the capillaries [67], stimulating fibroblast migration and proliferation and collagen organization, and at the final step accelerating wound healing [111].

4.2.2. Methylglyoxal (MGO)

MGO is produced from a non-enzymatic conversion of dihydroxyacetone (DHA) found in the nectar of the *Lepstospermum* flower at a slow rate during the honey maturation process [112]. The rates of conversion and the MGO concentrations found in manuka honey might depend on the storage temperature (usually 37 °C) when the conversion of DHA in MGO rapidly increases [112,113]. The concentrations of MGO in manuka honey are up to 100 times higher than in conventional honey [70]. This characteristic confers it a pronounced antibacterial activity, allowing it to be commercially classified as the best product, as shown via microbiological assays, with the marketing description of the "unique manuka factor" [114]. The lack of H_2O_2 does not compromise the bactericidal activity in manuka honey, showing MGO to be the major non-peroxide bactericidal factor [76]. MGO's mechanisms of action seem to be related to changes in bacterial fimbriae and flagella [92], which could explain the better effectiveness of manuka honey against *E. coli* and *B. subtilis*, limiting the bacterial adherence and mobility [75]. Moreover, MGO's mechanisms of action seem to be different in Gram-positive organisms, where it is able to impair cell division [77], while in Gram-negative bacteria MGO induced a loss of the structural integrity of the bacteria, as well as changes in cell shape and surface [77]. However, in patients with diabetes, treatment with high MGO concentrations [115] is concerning, since MGO is a powerful inductor of the formation of advanced glycation end products (AGEs), which are related to the disruption of collagen matrix remodeling, causing poor wound healing [116].

4.2.3. Phenolic Content

Chemically, phenolics are compounds that possess aromatic rings with one or more hydroxyl groups [117]. The presence of polyphenols was already recognized in food as an important factor in promoting microbiological safety [118]. In honey, polyphenols have been reported as non-peroxide antimicrobial elements [69], promoting antioxidant and anti-inflammatory properties, as well as conferring particular characteristics such as the color and flavor, which depend on the botanical origin [15,71]. The most available types identified in honey are phenolic acids (benzoic and cinnamic acids) and flavonoids (flavonols, flavones, and flavanones) [71]. To date, a review involving 536 monofloral honey types reported 161 different phenolic compounds, with caffeic acid, gallic acid, ferulic acid, and quercetin being the most prevalent in monofloral honey [119].

Notably, polyphenols can contribute to honey's antimicrobial activity by elevating the H_2O_2 levels [68]. Additionally, the presence of polyphenols in honey in low concentrations caused pro-oxidant activity, leading to the bacterial DNA being degraded, whereas in high concentrations the polyphenols were positively associated with bacteriostatic activity [120]. A honey sample with a higher total phenolic content (TPC) also presented higher ferric-reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant activity levels [121]. Furthermore, greater effectiveness against MRSA and *E. faecalis* was observed in honey with a higher TPC [122]. The addition of propolis to honey samples increased the amounts of phenolic compounds, which could partially explain the increased efficacy of this synergism against *S. aureus* and *E. coli* [62]. In fact, it seems that the TPC is directly associated with the DNA degradation extension [120] and negatively correlated with the minimum inhibitory concentration (MIC) [15]. Interestingly, a study compared the TPC values among Australian honeys and identified greater values for *Calothamnus* spp. (red bell) honey and *Eucalyptus marginata* (jarrah) honey when compared to the previously reported manuka honey, which is commonly stated to have greater antioxidant activity [121]. Similarly, a study compared Kazakhstani honeys and found that despite sunflower honey having a low TPC, high bactericidal activity was observed against MRSA [122].

These findings suggest that the phenolic content is not the major non-peroxidant antimicrobial factor in honey and that this antimicrobial activity might depend more on the polyphenols' composition than on their quantity [122]. Therefore, different phenolic compounds can confer different antibacterial characteristics to honey. For instance, caffeic

acid was reported to induce oxidative stress [123], whereas ferulic acid is more related to cell membrane damage, changing the cellular morphology [124]. Finally, gallic acid is also reported to compromise the cell membrane, leading to irreversible changes in its structure, as well as inducing pore formation [125]. Conversely, the compounds *t*-cinnamic acid, lumichrome, *o*-anisic acid, and eudesmic acid were associated with non-exertional antioxidant activity due to the lack of hydroxyl groups in their benzene ring [121].

4.2.4. Bee Defensin-1 (Def-1)

Bee-derived defensin-1 is an antibacterial peptide secreted in honey by the hypopharyngeal glands of honeybees [126,127]. Def-1 acts in bees as an innate immune response against microbial and fungal pathogens [127]. Due to its ability to induce DNA, RNA, and protein synthesis inhibition, as well as to decrease the bacterial viability, Def-1 has a crucial role in wound treatment, especially in biofilm destruction [128]. Despite Def-1 being effective against Gram-positive bacteria [129], controversial results are still observed in Gram-negative bacteria [39], possibly because they are resistant to insect defensins [130]. In the early biofilm stage, Def-1 might prevent biofilm formation, impairing the bacterial adhesion or inhibiting the growth of attached cells, as well as altering the production of extracellular polymeric substances [39]. In the wound healing process, Def-1 also acts by stimulating matrix metalloproteinase-9 (MMP-9) secretion from keratinocytes, as well as by enhancing the migration of keratinocytes in vitro and favoring wound closure and re-epithelialization in vivo [126]. An in vitro study showed that Def-1, together with H_2O_2, was involved in the rapid bactericidal activity against *B. subtilis*, *E. coli*, and *P. aeruginosa* in Revamil honey [76]. Moreover, the neutralization of Def-1 reduced the honey's effectiveness in the killing of vancomycin-resistant *E. faecium*, showing the bactericidal activity of Def-1 [79]. Despite this evidence, no correlation was found between the amount of Def-1 in honeydew honey and the bactericidal activity against *S. aureus* [68]. Different types of honey might present different Def-1 concentrations, since the amounts of bee gland secretion added to the honey may strongly vary [68,76].

4.2.5. Osmotic Effect

Honey is mostly composed of sugar and water, with sugars comprising 95–99% of honey's dry matter [107], classifying honey as a super-saturated solution of sugars [131]. In fact, the high sugar concentration found in undiluted honey was able to exert osmotic pressure on bacterial cells, killing them through osmosis [74,131]. Additionally, the interaction between sugars and water molecules decreases the free available water and impairs microorganism growth [131,132]. Despite these benefits, the current evidence shows that the osmotic effect contributes to honey's antimicrobial properties but that it is not the major factor. For instance, artificial honey showed effectiveness only against *P. aeruginosa* but not against *S. aureus* or *Streptococcus agalactiae* [39]. Honey's high osmolarity also led to an outflow of lymph, providing nutrition for the regeneration of tissue [88].

5. Types of Honey Effects

Since honeys have different characteristics, depending on the botanical origin, concentration, or storage conditions, it might be expected that different honey types possess different healing properties. Importantly, multi-floral honey exhibits a greater phenolic content than monofloral honey, which confers different levels of anti-inflammatory and bactericidal activity potential [133].

The topical application of manuka honey in streptozotocin-induced Wistar rats promoted a higher percentage of wound contraction and faster epithelization than a group treated with acacia honey (*Robinia pseudoacacia*) [134]. Similarly, Japanese acacia honey was not effective in promoting complete wound epithelialization [34]. Importantly, acacia honey is characterized by a lower polyphenol content and antioxidant capacity level among Romanian honey samples [135]. Interestingly, no differences were observed in wound

healing in comparisons between manuka and jamun honey (*Syzygium cumini*), which were characterized by similarly low pH levels and sugar and flavonoid contents [136].

Four hours after the topical application of different types of honey, namely manuka, kanuka, and rewarewa, in mice submitted to arachidonic-acidic-induced ear edema revealed that only rewarewa (*Knightea excelsa*) honey significantly reduced the edema and leukocyte infiltration when compared with the untreated negative control group [87]. Interestingly, the superoxide inhibition in vitro did not correlate with honey's phenolic content. The authors also reported an inverse correlation between manuka honey's MGO content and neutrophil superoxide production. However, this correlation was not caused by the MGO-dependent inhibition of the anti-inflammatory activity, suggesting the contamination of the manuka honey with nectar from other flower sources [87]. Similarly, Sidr honey (*Ziziphus spina-christi*) was the most effective treatment in thermal or chemical-induced wounds when compared to thyme (*Thymus vulgaris*) and spring honeys or conventional therapy [137], showing the diverse action of honey in wound healing.

The healing actions of different honeys might also differ according to the stage of wound healing. The in vivo application of acacia (*Robinia pseudoacacia*), buckwheat (*Fagopyrum esculentum*), and Chinese milk vetch (*Astragalus sinicus*) honeys was effective only in the inflammatory wound healing phase [34]. Conversely, a chestnut (*Castanea sativa*) honey hydrogel applied in the excisional wounds of *db/db* mice showed a delayed action in terms of wound closure during the first six days after wound excision [138]. Similarly, in streptozotocin-induced diabetic Wistar rats, wounds treated with mad honey only showed epithelialization and reductions in inflammatory cells similar to terramycin treatment at the 19th day, supporting a delayed mechanism of action in mad honey during the first days after wound excision [139].

In vitro comparisons between honey samples' bactericidal activity levels have shown that different types of honey have different mechanisms of action according to the different bacterial strains. For instance, Revamil medical-grade honey showed the highest antibacterial activity level against *E. coli* and *P. aeruginosa* after 2 h of incubation, whereas manuka honey was the most effective after 24 h. Against *B. subtilis*, manuka honey's bactericidal components such as MGO and its low pH were able to rapidly promote the bactericidal activity, similarly to Revamil honey after 2 h of incubation [76]. Furthermore, among the medical-grade honeys, Revamil was classified as the least effective, showing higher MIC levels, whereas Surgihoney 3 showed lower MIC levels against *S. aureus*, *MRSA*, *P. aeruginosa*, multidrug-resistant *P. aeruginosa*, *E. coli*, and *K. pneumoniae* [12]. Neither for Revamil nor for Surgihoney was information regarding the floral sources provided, hindering accurate comparisons between different types of honeys. A comparison between eight manuka honey and ten other medical-grade honey samples showed that against MRSA, multidrug-resistant *P. aeruginosa*, and *Staphylococcus epidermidis*, the Comvita Manuka 5+ honey exhibited the lowest MIC value among the manuka honey types in vitro [12].

The potential and particularities of healing with honey can also be related to honey's physical–chemical characteristics. For instance, it has been suggested from in vitro studies with Poland honey that the darkest honeys with strong yellow color components have higher antibacterial activity levels, especially against *E. coli* [15]. Similar findings were reported by Estevinho et al. [71]. According to the authors, the darkest honeys showed a higher phenolic content level and a good correlation with the antibacterial activity. Furthermore, the dark-colored honeys (e.g., linden and honeydew) showed higher mineral content levels than the light-colored honeys (e.g., acacia and wild cherries) [135]. Therefore, investigating the mechanisms of healing with honey based on its physical–chemical characteristics would be an interesting strategy to identify the best type of honey treatment for specific wounds or bacterial strains.

6. Honey in Diabetic Foot Treatment

Several studies carried out with animal models have shown the efficacy of honey in DW treatment [17]. In animal models of diabetes, honey is able to accelerate the wound

healing, promoting epithelialization, improving the tissue granulation, and increasing the wound contraction [136,138,139]. Streptozotocin-induced mice with diabetes treated with honey from the nectar of *Thymus serpillum* and *Astragalus microcephalus* showed reduced wound areas with greater wound contraction and epithelization than animals treated with isotonic saline solution [111]. The treatment with propolis honey hydrogel dressings derived from Seoul Propolis Co., Ltd. (Daejon, Korea), in *db/db* female mice was effective against *S. aureus* and *E. coli* and improved the amounts of wound area reduction and contraction [41]. The topical application of manuka (*Lepstospermum*) and jamun (*Syzygium cumini*) honeys in animals with diabetes not only accelerated the wound closure and re-epithelialization but also improved the collagen deposition and modulated the essential angiogenic markers, namely hypoxia-inducible factor and vascular endothelial growth factor [136].

In diabetic mice, heme oxygenase-1 (HO-1), which is a cytoprotective, pro-angiogenesis, and anti-inflammatory enzyme, is usually found to decrease, impairing the regulation of angiogenesis [140]. The application of a chestnut (*Castanea sativa*) honey hydrogel in db/db mice was able to rapidly upregulate HO-1 proteins at the wound site, which might mediate the coordination of keratinocytes and enhance the expression of Ki-67 proliferation markers [138]. The topical application of mad honey, a *Rhododendron* honey containing grayanotoxins, also decreased the gene expression of inflammatory markers such as TNF-α and metalloproteinase 9 (MMP-9) and increased the IL-10 expression [139]. According to the authors, the honey's characteristics, such as its optimum phenolic and flavonoid contents, were responsible for the mad honey's antioxidant properties. The information from experimental studies involving models of diabetic animals treated with honey is summarized in Table 1.

In humans, honey has been shown to be effective in DFU treatment, decreasing the bacterial load and inflammation, regenerating the granulation tissue, and reducing the wound size, as well as the rate of amputation [141–144]. A clinical study over 16 weeks carried out in 63 patients with DFU treated with manuka-honey-impregnated dressings showed accelerated rates of wound healing and disinfection, as well as a nullified need for antibiotics or hospitalization, in comparison with conventional saline-soaked dressings [110]. Another clinical trial compared the DFU treatments from debridement to wound closure in 33 patients and found a higher wound healing rate in those treated with a thin layer of Australian honey dressings covered with gauze (mean: 14.4 days; range: 7–26) than with povidone-soaked gauze (mean: 15.4 days; range: 9–36) [145]. A reduced wound size and higher wound healing rate were observed in patients submitted to beri (*Ziziphus jujuba*) honey dressings (n = 136; median 18 days) than in patients treated with saline dressings (n = 97; median 29 days) [146]. Currently, five clinical cases have exhibited the effectiveness of medical grade honey in reducing the wound area, exudate volume, and severity of infection, as well as wound-related pain, in diabetic patients [147]. Allergy and irritation symptoms were also decreased in DFU patients treated with Jordanian natural honey [142]. Despite these benefits, in cases of severe vascular compromise, exposed bone, or established osteomyelitis, honey was not as effective [19]. The characteristics of the studies carried out with human DFUs treated with honey are detailed in Table 2.

Table 1. General characteristics of studies where diabetic animals were treated with honey.

Authors (Country)	Animal Models	Reagents Time of Intervention	Honey Characteristics	Results
Malkoç et al., 2020 (Turkey) [139].	Streptozotocin- induced diabetic male Wistar rats. (n = 84) 8–10 weeks old	Mad honey vs. terramycin vs. 0.09% saline solution Intervention time: 19 days	Mad honey *(Rhododendron)* Color: nr Moisture: 18.69% pH: 5.20 Glucose: 27.30% Fructose: 34.80% TPC (mg GAE/100 g): 33.5	Mad honey and terramycin showed higher wound contraction mean ($p < 0.05$), higher IL-10, and lower TNF-α and MMP-9 gene expression values than saline solution. Mad honey had lower malondialdehyde levels ($p < 0.05$)
Chaudhary et al., 2020 (India) [136]	Streptozotocin- induced diabetic male Swiss albino rats. (n = 60) 8–12 weeks old	Jamun honey vs. manuka honey vs. povidone–iodine Intervention time: 30 days	Manuka (Medical grade honey) Color: light amber Water content: 10.76% pH: 3.93 Total sugar content: 86.19% TPC (mg GAE/100 g): 256.6 Jamun honey (*Syzygium cumini*) Color: amber Water content: 14.06% pH: 3.46 Total sugar content: 84.55% TPC (mg GAE/100 g): 389.34	Jamun-honey- and manuka-honey-treated wounds had higher wound closure rates than with povidone iodine for both diabetic and non-diabetic mice ($p < 0.05$). HIF-1a, VEGF, and VEGF R-II were upregulated after both honey treatments ($p < 0.05$). No differences between the types of honey were found.
Gill et al., 2019 (India) [134].	Streptozotocin- induced diabetic male Wistar rats. (n = 42) nr	Manuka honey * vs. acacia honey * vs. 2% *w*/*w* sodium alginate gel vs. silver sulfadiazine cream Intervention time: 21 days	Manuka honey (*Leptospermum scoparium*) Acacia honey (*Robinia pseudoacacia*)	Manuka honey caused ≥80% wound contraction at day 9 whereas acacia honey caused around 60% for diabetic and non-diabetic mice. Healing status: Poor: sodium alginate gel; fair: acacia honey; good: manuka honey.
Rashidi et al., 2016 (Iran) [148].	Streptozotocin- induced diabetic male Wistar rats. (n = 42) nr	Nika cream vs. phenytoin 1% vs. non-treated Intervention time: 24 days	Nika cream: mixture of honey, royal jelly, and olive oil–propolis extract (*Olea europaea*).	Nika cream caused accelerated wound closure in comparison with phenytoin 1% and non-treated control, respectively.

Table 1. *Cont.*

Authors (Country)	Animal Models	Reagents Time of Intervention	Honey Characteristics	Results
Nho et al., 2014 (Korea) [41]	Diabetic (db/db) female mice (n = nr) 5 weeks old	Honey-carboxymethyl cellulose hydrogel vs. carboxymethyl cellulose hydrogel vs. no treatment Intervention time: 15 days	Propolis honey from Seoul Propolis Co., Ltd. (Daejon, Republic of Korea)	Honey-carboxymethyl cellulose hydrogel caused a higher wound contraction rate than in other groups.
Choi et al., 2012 (Korea) [138]	Diabetic (db/db) male mice (n = 84) 10 weeks old	Chestnut honey hydrogel vs. water hydrogel vs. non-treated control Intervention: 15 days	Chestnut honey (*Castanea sativa*) Dark color	Higher wound closure rate caused by chestnut honey hydrogel than water hydrogel.
Demir et al., 2007 (Turkey) [111]	Streptozotocin- induced diabetic male Swiss albino rats. (n = 27)	Honey vs. isotonic sodium chloride Intervention: 9 days	Thyme (*Thymus serpillum*) and Astragalus (*Astragalus microcephalus*)	Honey caused higher wound contraction and epithelialization rates.

Note: DM: diabetic model; G: group; N: sample number; NDM: non-diabetic model; nr: not reported; TPC: total phenolic content; W: wound; * manuka and acacia honey were applied in the condition with 10% or 15% concentration added to 2% *w*/*w* sodium alginate gel.

Table 2. General characteristics of studies in which DFUs in humans were treated with honey.

Authors (Country)	Sample Profile DFUs Stage	Treatments	Honey Characteristics	Results
Holubová et al., 2023 Czech Republic [147]	Patients with diabetes (n = 5) Age: 61.6 years nr	Medical grade honey	nr	Wound reduction, healing, and infection control. Reduced exudate secretion, odor, and wound-related pain.
Agarwal et al., 2015 (India) [149]	Non-insulin-dependent diabetes (n = 36) Age: 52.4 ± 5.4 years Wagner grade II	Honey vs. povidone iodine solution 10%	ns	Honey wound healing time was 14.2 days vs. 15.5 days for povidone–iodine ($p > 0.05$). Honey treatment reduced pain, edema and foul-smelling discharges when compared to povidone–iodine.
Imran et al., 2015 (Pakistan) [146]	Patients with diabetes (n = 375) Age: 54 (47–64) years * Wagner grades I and II	Honey vs. saline dressing	Beri (*Ziziphus jujuba*) honey	In total, 75.97% of patients treated with honey showed completely healed wounds vs. 57.39% with saline dressings. Honey rate of healing time was 18 (6–120) days, whereas the time for saline dressings was 29 days (7–120) days ($p < 0.001$). *

Table 2. *Cont.*

Authors (Country)	Sample Profile DFUs Stage	Treatments	Honey Characteristics	Results
Surahio et al., 2014 (Saudi Arabia) [141]	Patients with diabetes (n = 172) Age: 25–70 years nr	Honey	ns	Wounds were healed within a range of 7–35 days.
Al Saeed et al., 2013 (Saudi Arabia) [144]	Patients with diabetes (n = 59) Age: 55 ± 13 years Wagner grade II, III and IV	Honey vs. tulle grass dressings	Manuka honey UMF 15	Infections treated with honey were more rapidly eradicated than with tulle grass ($p < 0.05$). In six weeks, 61.3% of patients treated with honey completely healed versus 11.5% treated with tulle gass ($p < 0.05$).
Kamaratos et al., 2012 (Greece) [110]	Type II diabetic patients (n = 63) Age: 56 ± 14 years Wagner grades I and II	Honey dressing vs. saline-soaked gauze dressings	Manuka (*Leptospermum scopar- ium*) honey	Wounds treated with honey healed in 31 ± 4 days, whereas those treated with saline-soaked gauze healed in 43 ± 3 days ($p < 0.05$). No patients treated with honey needed antibiotics, whereas 9% of those treated with saline-soaked gauze needed further treatment.
Jan et al., 2012 (Pakistan) [143]	Patients with diabetes (n = 100) Age: 56 ± 8.0 years Wagner grades I to IV	Honey vs. conventional pyodine	ns	In total, 60% of patients healed with honey within 2–4 weeks, 34% in 5–7 weeks, and 6% in 8–10 weeks. With pyodine, 30% healed within 2–4 weeks, 26% in 5–7 weeks, and 44% in 8–10 weeks.
Shukrimi et al., 2008 (Malaysia) [145]	Non-insulin-dependent diabetes (n = 30) Age: 35–65 years Wagner grade II	Honey vs. povidone iodine solution 10%	Australian honey (ns) pH: 6.5 Glucose: 321 mmol/L Specific gravidity: 1.003	The mean wound healing period with honey treatment was 14.4 days, whereas with povidone–iodine it was 15.4 days ($p < 0.005$). Honey treatment improved edema symptoms and foul smells and the patients experienced less pain than with povidone–iodine.
Hammouri et al., 2004 (Jordan) [142]	Patients with diabetes (n = 200) Age: 22–100 years nr	Honey vs. povidone iodine and hydrogen peroxide at a ratio of 3:1	Jordanian natural honey	The mean honey wound healing period was 21 days, whereas for povidone–iodine it was 32 days ($p < 0.001$). Hospitalization and amputation rates decreased in 43% and 50%, respectively, of patients treated with honey ($p < 0.05$). Povidone caused higher irritation and allergy rates from treatment ($p < 0.001$).

Note: DM: diabetic model; NDM: non-diabetic model; N: sample size; nr: not reported; ns: not specified; G: group; TPC; UMF: unique manuka factor; * data in median and interquartile ranges.

Finally, two recent meta-analyses showed that the use of honey dressings was associated with higher wound healing and bacterial clearance rates after one week and two weeks of treatment, respectively [150], as well as accelerated granulation and reductions in hospitalization and incurred pain [151]. Moreover, the use of honey was associated with shorter bacterial clearance, wound debridement, and wound healing time periods when compared to other dressing types [150]. All of this evidence supports the notion that honey is a suitable and promisor biomaterial for wound healing, especially for DFU treatment.

7. Conclusions

Despite meaningful advances in diabetes treatment, DFU management is still considered a public health challenge. Multidrug-resistant microorganisms represent considered one of the worst concerns in DFU treatments, leading to greater rates of lower-limb amputation. Honey has been shown to be a cost-effective treatment option for DFU management, mainly due to its antioxidant, anti-inflammatory, and antibacterial potential, including against several antibiotic-resistant bacterial strains. Peroxide and non-peroxide mechanisms are responsible for promoting wound healing. Furthermore, honey's physical–chemical characteristics, particularly its high osmolarity, low pH, and low moisture levels may promote a suitable environment to accelerate the wound healing and decrease the amputation and hospitalization rates. This paper has presented the current evidence regarding how honey dressings can contribute to DFU treatment via different mechanisms. In vitro studies have shown the antimicrobial effectiveness of honey against multidrug-resistant bacteria and in vivo studies conducted with diabetic animal models have shown accelerated wound healing and infection control in animals treated with honey dressings. Most clinical studies conducted with humans have found accelerated DFU healing and infection control rates and reduced hospitalization and amputation rates. Despite the available evidence, few experimental studies describe honey's characteristics and how these specificities might correlate with the different findings regarding the clinical effectiveness of different honey types. Moreover, the lack of information regarding pollen profiles hinders accurate comparisons being made between types of honey.

Author Contributions: A.B.: Writing—original draft preparation, review and editing. H.F.: Writing—review and editing, supervision. F.R.: Writing—review and editing, funding acquisition. C.D.-M.: Writing—review and editing, funding acquisition. I.G.: Conceptualization, writing—review and editing, supervision. J.G.: Conceptualization, writing—review and editing, supervision. All authors have read and agreed to the published version of the manuscript.

Funding: This work received financial support from national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through project MTS/SAS/0077/2020: "Honey+—New Reasons to Care About Honey from the Natural Park of Montesinho: A Bioindicator of Environmental Quality and its Therapeutic Potential" and through the project UIDB/50006/2020. This research was also funded by the Center for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB) research unit, grant number UIDB/04033/2020.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Andréa Bezerra thanks FCT (Fundação para a Ciência e Tecnologia) for funding through the Honey+ project (MTS/SAS/0077/2020) and its fellowship BI/UTAD/42/2022. Francisca Rodrigues thanks FCT (Fundação para a Ciência e Tecnologia) for funding through Scientific Employment Stimulus—Individual Call (CEECIND/01886/2020). Irene Gouvinhas thanks FCT (Fundação para a Ciência e Tecnologia) for funding through Scientific Employment Stimulus—Individual Call (2022.00498.CEECIND). Juliana Garcia is grateful for funding support from Portuguese public funding through Investimento RE-C05-i02–Missão Interface N.° 01/C05-i02/2022 and from FCT for the projects titled "AquaValor—Centro de Valorização e Transferência de Tecnolo-

gia da Água" (NORTE-01-0246-FEDER-000053) and "Emprego altamente qualificado nas empresas ou em COLABS—Contratação de Recursos Humanos Altamente Qualificados (PME ou CoLAB)" (NORTE-06-3559-FSE-000095), supported by the Norte Portugal Regional Operational Programme (NORTE 2020) under the PORTUGAL 2020 Partnership Agreement through the European Regional Development Fund (ERDF).

Conflicts of Interest: The authors declare no conflict of interest.

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