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Topical Saudi Arabia Talh honey (*Acacia nilotica*) on surgical wound healing activity

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Abstract

Honey has been documented as the oldest traditional medicine. It has been effective in suppressing inflammation, wound repair enhancer, and rapid autolytic debridement. The aim of this investigation was to evaluate the role of Saudi Arabia Talh honey (*Acacia nilotica*) dressing as a good alternative in care of diabetic foot (DFU) healing activity for twenty patients, wound total bacterial count, and serum cytokines levels (IFN- γ , IL-1, and IL-6). The results showed that Talh honey stimulates the wound healing process, broad-spectrum antibacterial activity, and reduction in the proinflammatory cytokines IFN- γ , IL-1, and IL-6 levels. It could be concluded that Talh honey bioactivities enhance wound healing by promoting tissue growth leading to wound repair, antibacterial, and reduction of inflammation.

Keywords: Honey, wound healing, cytokines, total bacterial count.

Introduction

One of the emerging options is the honey dressing due to a variety of bioactive compounds found in honey [1], which has broad-spectrum bactericidal effects [2], reduces inflammation and promotes debridement [3], immune cell activation [1] and reducing bad odor [4]. Using the topical application of honey in superficial and partial thickness burns was performed to compare film and gauze-based dressings [5]. Brölmann *et al.*, [6] found that honey was effective in dressings, local debridement therapy, effective in reducing wound healing time and systemic antibacterial therapy. The main reasons for morbidity in particular, diabetic patients referred to, foot ulcers, which reached 50% of non-traumatic amputations. Also, the management of diabetic foot ulcers is an important integral part of wound dressing [7].

Improve health and spur economic growth are the heart of Sustainable Development Goals (SDGs). Therefore, to induce these improvement uses the natural products. The randomized clinical study required one of the best alternatives natural products as honey to compare in wound care and a variety of existing topical agents. Previously, some clinical trials reported to evaluate honey effects in wound healing, particularly diabetic foot ulcers. Therefore, this study aimed to determine the role of Saudi Arabia Talh honey (*Acacia nilotica*) in the diabetic foot (DFU) wound care as a dressing with a good alternative in treating advanced wounds.

Materials and Methods

Ethical approval

All experimental procedures were carried out under Protocol No. 20072, in accordance with the guidelines of the National Research Centre, Dokki, Giza, Egypt.

Experiment Design

Twenty patients from General Surgery out clinic, this study was conducted. It was a 3-year, prospective, RCT which was started from 2018 to 2020. The data collection and pro forma were filled either by the patient or relatives. All patients ≥ 18 years of age were selected for this study with postoperative wound, diabetic foot ulcer. Diabetic foot ulcer patients: Ankle Brachial Pressure Index (ABPI) < 0.7 , uncontrolled diabetes i.e. HbA1c $> 7\%$, hemoglobin 10 g/dl also, local signs of infection were the presence of pus and culture-positive were included. The subjects were divided into two groups (ten each). The first group was treated with fresh acacia Talh (*Acacia nilotica*) while the second group treated with conventional treatment. To remove debris, the wound was washed before dressing with usual saline and patients were admitted to the hospital for at least the first 2 dressings, protective dressing was sealed with the second layer and depending on the wound condition the dressing was done once or twice daily for each day, where the dressing was done by doctor or nursing staff as a dressing procedure in the out-clinic. According to the situation, loading was done by using special full-boot cast, or shoes, or crutches. When required debridement of the wound was done. It was performed in the outpatient department under local anesthesia or without anesthesia if the patient did not feel pain.

The Visual Analog Scale [8] was performed for evaluation of wound healing activity. Complete healing of the wound was the primary outcome. While the secondary outcomes were considered as the satisfaction of patients', side effects of dressing methods and healing time, side effects of dressing methods, signs of local or systemic infection deterioration of wound. Up to 120 days or earlier if the wound was healed, all participants were monitored. Complete epithelialization, no discharge, and wound closure were considered to be wound healing.

Under aseptic condition swabs were taken from the wounds for bacterial examination was performed. The serum cytokines (IFN- γ , IL-1, and IL-6) levels were evaluated before and after treatment.

Acacia Talh (*Acacia nilotica*) honey (30 kg), 2018 flowering season was kindly provided by Alnahal aljwal Company, Saudi Arabia. The physiochemical analysis was performed according to Hegazi *et al.*, [9]. The consideration of fresh monofloral honey was performed as described by Louveaux *et al.*, [10]. Honey was stored at $2-8^\circ\text{C}$ in a sterile glass container until used.

Laboratory Analysis

Determination of bacterial count (CFU): Under aseptic condition swabs from wounds were taken. These swabs were incubated in 10 ml of nutrient broth at 37°C for 24. Then 10-fold serial dilutions of the homogenates in the nutrient broth were cultured, in duplicates dilutions were cultured on neomycin blood agar using $100 \mu\text{l}$ of the diluted specimen for each plate [11]. Plates were incubated for 24-48 h at 37°C on $5\% \text{ CO}_2$. By using a colony counter, the bacterial colonies

were counted as the method described [12]. The colonies of isolated bacteria were confirmed microscopically and biochemically as described in the method of [13].

Serum cytokine levels: At before and after treatment at the end of the study interleukin (IL) 1β , IL-6, and IFN- γ , were assessed using sandwich ELISA [14]. The serum cytokines levels were measured by using commercially available ELISA kits according to the manufacturers' directions (kits purchased from Biovision Co., China).

Statistical analysis

The results reported in this study have been interpreted as a means \pm standard error, and analyzed using variance analysis (ANOVA). Samples were analyzed using Student's t-test (two-tailed) for unpaired samples with equal variance using Excel (Microsoft, Seattle, WA). Using the Duncan Multiple Range Test, the significance of the difference between mean values at $P < 0.05$ was calculated.

Results

In this study, honey has been used to treat with a number of chronic, acute, infected, or badly colonized wounds. The latter wounds, however, remained free of infection, and displayed no further deterioration. All patients required systemic antibiotics. It was noted that showed honey within 24 hours has a rapid cleansing and deodorizing action also reduced inflammation and exudate levels particularly on fungating (malignant) wounds (**Figure 1**).

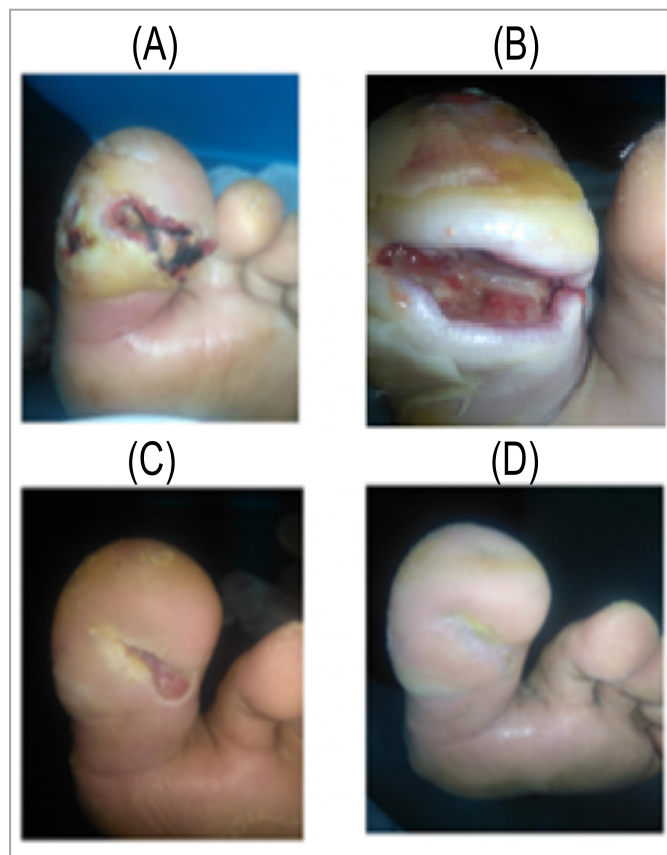


Figure 1. Randomized controlled trials of honey. (A) gangrenous part of wet type of diabetic foot need to mechanical debridement. (B) Wound with little necrotic tissue after dressing by honey. (C) Wound decrease in size. (D) wound covered by skin and crustation.

The following randomised controlled honey experiments showed good results as compared with other dressing strategies: (1) Honey compared to povidone-iodine, (2) Honey compared to povidone-iodine with 70% ethanol washes on extreme post surgical wound infections, and (3) Honey compared to regular methods such as normal saline with glycerin amnesia or creams (mebo, iruxol, panthenol).

Quantification of patient’s sera cytokines

Honey therapy demonstrated a substantial change in immunological status as a result of a decline in inflammatory serum cytokines (Figure 2). It was clear that there was a significant (p<0.05) reduction in serum, IFN-γ (-12%), IL1β (50%), and IL6 (-55%) relative to the corresponding pre-treatment values.

Evaluation of the antibacterial activity of Saudi Arabia Talh honey (*Acacia nilotica*)

The investigations of this microorganism during this study indicated that the isolated bacteria were characteristic by numerous short, thick, straight, round-ended, cocci Gram-positive, and Gram-negative identified in agar plates used for subculture. The total bacterial count was 8×10^9 at the beginning of the experiment then it reduced after the application of traditional therapy to (9×10^4) while honey treatment reached to (9×10^2) after 17th days post-treatment as shown in Figure 3.

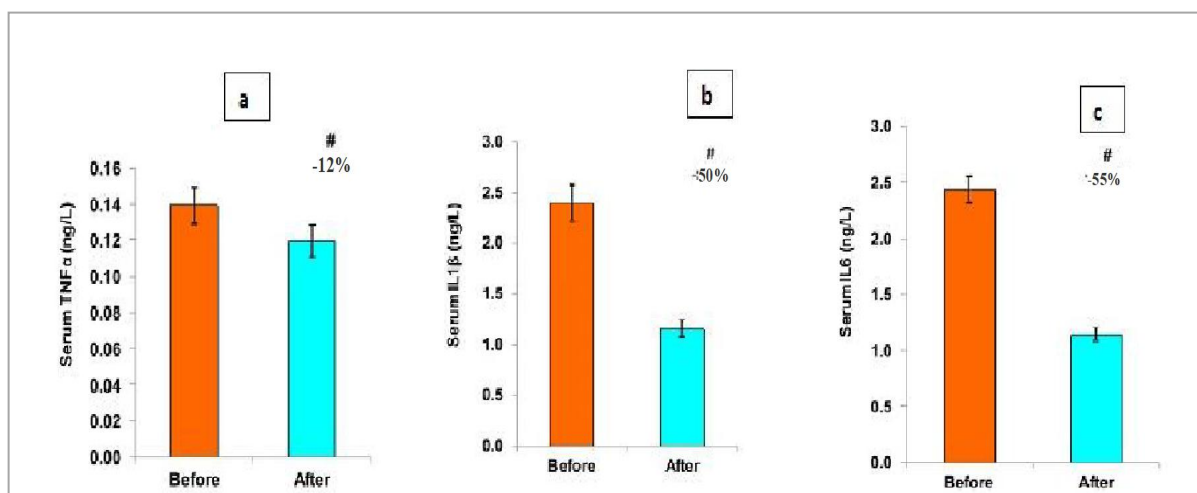


Figure 2. The effect of the honey treatment on patients serum level of (a) IFN-γ, (b) IL1β, and (c) IL6.

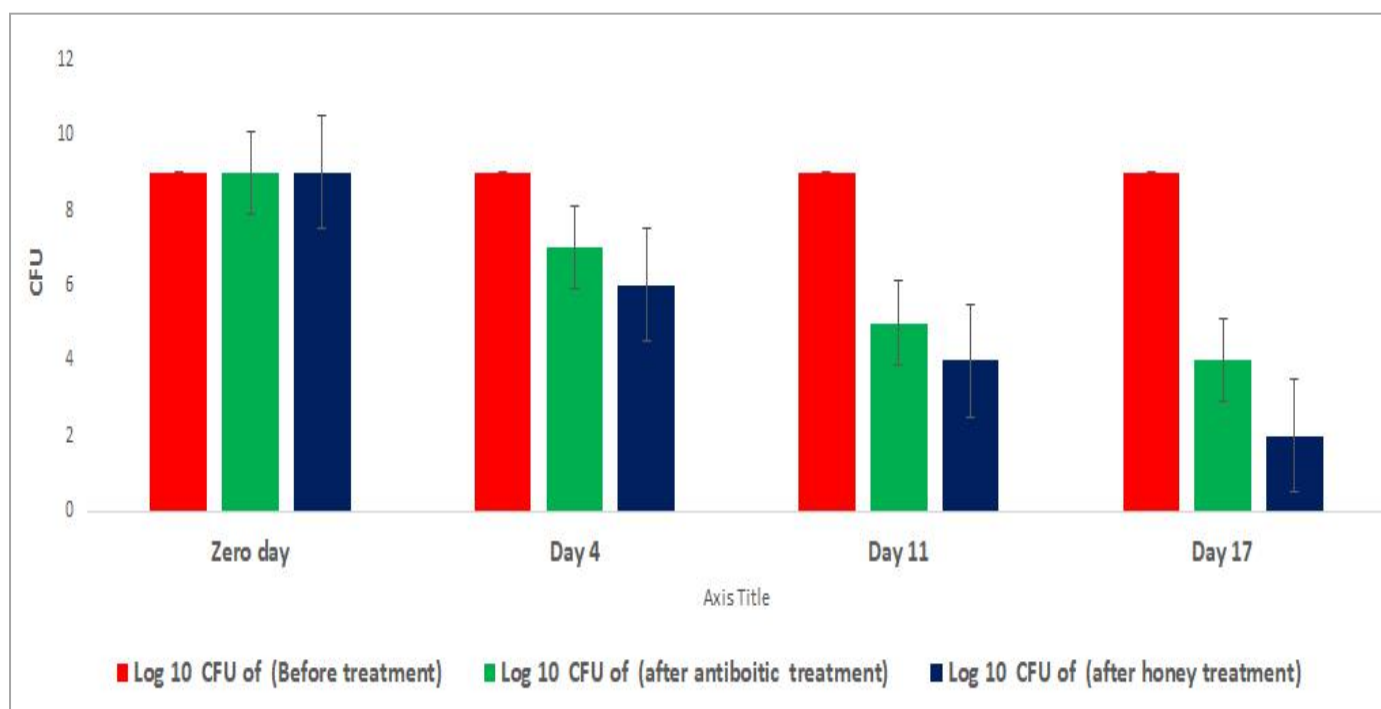


Figure 3. Total bacterial count (Log10) before and after treatment with Saudi Talh honey.

Discussion

Honey dressing in the current clinical trial was more effective than regular saline and povidone-iodine dressing in the management of post-operative wounds and diabetic foot ulcers. The reasons for this outcome could be due to honey has growth factors, potent debriding effects, antibacterial activity, and anti-inflammatory. Also, honey is helpful for the removal of toxins through increases in lymph flow in the wound. It was reported in a clinical study on the positive effect of honey dressing in post-surgical wounds.

Moore *et al.*, [15] found that honey has healing activity and a shorter time than all these treatments used in this aspect. Also, Wang *et al.* [16] stated that honey more effective, easy application, better outcome, and patients' satisfaction for the treatment of diabetic foot ulcers. Moghazy *et al.*, [17] studied honey in clinical and cost-effectiveness in the treatment of post-operative and diabetic foot ulcers. In the current study, patients were good assessment and the study continued for 3 years. It was observed that it was appropriate for treated subjects that belonged to a lower socio-economic class, and that wound healing was observed only clinically, and isolation of microorganisms to estimate the total bacterial count. It was observed that honey stains the wound margins and it has a specific odour and colour. Bowler *et al.*, [18] showed a lack of facilities belonged to lower socioeconomic class. It was clear that there was a significant reeducation after using natural honey also reeducation in the dressing budget reached 75% as well as patients' pain levels as evidenced by the use of the Visual Analog Scale. Although effectiveness of honey dressing in post-operative wounds and diabetic foot ulcers were under taken in several studies. On the other hand, many clinicians are still hesitant to use it in their clinical practice [3]. These results may be due to many reasons, such as lack of a clear level one proof of the positive effect of honey on wound ulcers, personal bias, and lack of knowledge of the full spectrum of honey antibacterial and wound healing ability [3]. Efficient honey dressing agent instead of traditional dressings in the treatment of diabetic foot ulcer patients determined in our results of this study in accordance with the findings of Imran *et al.*, [1]. Shukrimi *et al.*, [19] reported that honey was a safe alternative treatment for Wagner grade II diabetic foot ulcers. Cooper [20] stated that debriding, anti-inflammatory characteristics, the ability to promote angiogenesis and the stimulation of healing were included as therapeutic claims.

Results of the current investigation using honey leads to wound healing due to its stimulation of inflammatory cytokines from monocytic cells these results confirmed by previous findings of Tonks *et al.*, [21] who found that effective role in healing and tissue repair conducted out by monocytic cells. Also, honey able to stimulate leukocytes to release cytokines [21], which initiates the tissue repair process [22]. Honey also activates the immune response to infection [23], other aspects of the immune system [3], also

B- and T- lymphocyte proliferation and the action of phagocytes [24]. Honey triggers the production of antibodies [25]. It has the improvement of the immune system and antibacterial activity [26]. Many authors as Mandal and Mandal [27], Hegazi *et al.*, [2,9] and Kalakattawi *et al.*, [28] detected broad-spectrum antibacterial activity of honey.

This investigation was conducted to evaluate the pro-inflammatory cytokines IFN- γ and interleukin (IL) -1 β , and IL-6) at baseline and after 6 weeks of the end of the study using enzyme-linked immunosorbent assays. Results are shown as the mean \pm standard error in pictograms per milliliter. All patients with diabetic foot in the group treated with honey had significantly higher cytokine concentrations vs control Group using the traditional treatment. Subjects at the end of the experiment were significantly reduced (**Figure 2**). Similar findings showed an alteration of various cytokines in patients enduring diabetic foot ulcers [29]. Nuwa *et al.*, [30] and Zubair and Ahmad [31] reported that the diabetic wound healing process can be accelerated by controlling cytokines levels.

In the present study, the total bacterial count was 8×10^9 at zero time, then gradually reduced to reach (9×10^4) after the application of traditional therapy and (9×10^2) in honey treatment after 17th days post-treatment. This result proved significant antibacterial activities of honey, which is probably attributed to

The antibacterial activity of honey refers to many factors as observed with many authors [32-35]. The antibacterial activity of different honey was previously studied by and Hegazi *et al.*, [2,9] and Cooper [29]. In any wound, the abundance and variability of microorganisms would be influenced by various factors such as type of wound, depth, location and quality, tissue level and immune response of the host [18]. A wide variety of microorganisms' growth in the wound becomes optimal if the involved tissue devitalized, contaminate, colonize, and the low host immune response [18]. *Staphylococcus epidermidis*, micrococci exposure as one of the members of the normal skin microflora of devitalized tissue associated which leading a slowly chronic wound healing to facilitate colonization and establishment of a wide variety of endogenous microorganisms. Sufficiently hypoxic in wounds will not reduce the susceptible environment to a wide variety of endogenous anaerobic bacterial colonization [36]. Whereas minor wound healing may provide sufficient time for a relatively low number of skin contaminants to reside [37], the continued exposure of damaged tissue tissue correlated with slowly healing chronic wounds is likely to promote colonisation and the establishment of a wide range of endogenous microorganisms [38].

Conclusion

The findings of the current investigation may be concluded that honey dressing plays a significant role in healing ulcers and time for healing compared to conventional

dressing substances in diabetic foot ulcers. Honey has antimicrobial activity and its growth factors influence healing. As well as being a natural product, honey has a variable in its chemical composition and biological activities.

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