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


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The effect of Qatada (*Acacia hamulosa*) honey from Saudi Arabia on the steps of pilonidal sinus healing

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Abstract

The purpose of this study was to provide more light on the effect of Qatada (*Acacia hamulosa*) honey from Saudi Arabia as a dressing on pilonidal sinus wound healing, antibacterial activity, and cytokine levels. The results revealed that the pH of 3.56 of tested honey, where glucose was 378 mmol/l also the specific gravity was 1.435, while contents of total phenolic and flavonoid and DPPH activity (32.15 mg GAE/100 g honey), (54.27 mg RE/100 g honey) and (127.83 mg/ml) respectively. The mean duration of pilonidal sinus wounds to be ready for surgical closure was 43.5 days in the control (standard dressing) group where the honey dressing group was 28.5 days. Less pain, edema, and foul-smelling discharge were expressed in the honey group if compared with the standard dressing group. The total bacterial count was 9×10^9 at the begging of the experiment then it reduced after the application of standard dressing traditional therapy to (9×10^5) and honey treatment group (9×10^2) after 21 days post-treatment. Significant reduction ($p < 0.05$) was observed in serum TNF α (-52), IL6 - 46), and IL-17 (-60) relatively to pre-treatment values. Based on our findings, we may conclude that Qatada honey bioactivities aided wound healing by increasing tissue growth while decreasing overall bacterial count and inflammation.

Keywords: Antibacterial activity, Cytokines, Pilonidal sinus, Honey, Healing

Introduction

For thousands of years, Humans had used honey due to its medical importance as well as it is mentioned in all religions [1]. It has been used in folk medicines as a remedy for many diseases [2]. Qatada honey is produced by collecting nectar from *Acacia hamulosa* plant flowers grown in Saudi Arabia. Its biological properties (antimicrobial and antioxidant capacities) have been deserving special attention [3]. Acacia honey has potential biological [4] and health activities which include antioxidant [5], immuno-modulatory [6], antiproliferative [7], neurological properties [8], reproductive [9], antioxidant [10], anti-microbial (11,12), wound healing [13] and anti-inflammatory [14]. Saudi Arabia honey was studied by many authors as (12,15,16). For thousands of years, Humans had used honey due to its medical importance as well as it is mentioned in all religions [1]. It has been used in folk medicines as a remedy for many diseases [2].

Qatada honey is produced by collecting nectar from *Acacia hamulosa* plant flowers grown in Saudi Arabia. Its biological properties (antimicrobial and antioxidant capacities) have been deserving special attention [3]. Acacia honey has potential biological [4] and health activities which include antioxidant [5], immuno-modulatory [6], antiproliferative [7], neurological properties [8], reproductive [9], antioxidant [10], anti-microbial [11,12], wound healing [13] and anti-inflammatory [14]. Saudi Arabia honey was studied by many authors as [12,15,16]. A pilonidal sinus (PNS) is a small hole or tunnel in the skin [17]. It causing the formation of a cyst or abscess. it becomes infected, it may ooze pus and blood and have a foul odor [18]. The lesions may contain hair and skin debris [19]. Three per 10,000 people per year had an incidence of affection. The occurrence in females less often in males while the young adults are commonly affected [19].

Treatment mainly performed by treating the area with an antiseptic [20], as an iodine-based solution, and after making a small incision in the skin by using a sterile sharp needle or lancet to allow the pus fluid to drain out through the incision [21]. The novelty of this research done due to antibiotic drug resistance; this is needing a natural product to apply such a case as pilonidal sinus. Many researchers did humans use honey for diabetic ulcers and surgery [11, 12, 22]. This study through more light on the effect of Qatada (*Acacia hamulosa*) from Saudi Arabia on pilonidal sinus wound healing, antibacterial activity, and cytokines levels.

Materials and Methods

Ethical approval

All experimental procedures were carried out in accordance with guidelines Protocol No. 20072 of National Research Centre, Dokki, Giza, Egypt. Ten female patients from the clinic of the medical service unit suffering from pilonidal sinus were selected in a randomized controlled clinical trial with two parallel arms (September 2019 - February 2020) with written consent after approval of the Medical Ethical Committee. The effects of topical application of Qatada (*Acacia hamulosa*) honey from Saudi Arabia on patients suffering from pilonidal sinus were intended in this study.

Selection criteria

The clinical trials included patients ranging in age from 55 to 72 years. Exclusion criteria included patients receiving steroid therapy, having a neutrophil count of less than $2000/\text{mm}^3$, and having multiple medical co-morbidities.

Treatment protocol

In this study randomized patients were conducted; standard dressing (normal saline used as a diluent of povidone-iodine solution 10%) and honey dressing (fresh Saudi Arabia Qatada (*Acacia hamulosa*) groups. All patients resident with at least 2 months of surgical experience, received appropriate antibiotics and the pilonidal sinus was debrided surgically. During the debridement and under aseptic conditions swabs were taken from the wounds for bacterial culture sensitivity. The optimum profile of blood glucose was kept. A trained nurse has carried out wound dressing started on the first post-observation day. Initial cleaning of the wound with normal saline was done. On the wound thin layer of honey then covered with sterile gauze and a bandage was applied in the honey dressing. While the wound was first cleaned with normal saline, it the then covered with povidone-soaked gauze in the control (standard dressing). All wounds blindly assessed every day by a surgeon with the material of dressing. Wound assessment ended either ready for surgical closure or needed further debridement. the swab was taken from the wound for culture and sensitivity testing when the assessing surgeon declared the wound was ready for closure. The Visual Analog Scale [23] was performed for evaluation of wound healing activity. Complete healing of the wound was the

primary outcome. While the secondary outcomes were considered as side effects of dressing methods and healing time, side effects of dressing methods, signs of local or systemic infection deterioration of wound, and satisfaction of patients. If the wound was healed, up to 60 days or earlier, all participants were monitored. Complete epithelialization, no discharge, and wound closure were considered to be wound healing. From all patients in both groups, blood samples (5 ml) were withdrawn from each patient to determine serum cytokines levels ($\text{TNF}\alpha$, IL6, and IL-17) before and after treatment.

Honey used

Fresh Saudi Arabia monofloral Qatada (*Acacia hamulosa*) honey sample (5 kg) was obtained from, 2019 flowering season From Alnahal aljwal, Saudi Arabia. At $2-8^\circ\text{C}$ the honey sample was kept until tested in a sterile universal glass container. Collected honey contents of total phenolic, total flavonoid, and free radical scavenging activity as well as physicochemical properties were assessed.

Serum cytokine levels

From each patient withdrawn 5ml of blood samples to separate sera samples were done. The sera samples were divided into aliquots and stored at -70 till used for assessment of the $\text{TNF}\alpha$, IL6, and IL-17 by ELISA kits (kits purchased from Biovision Co., China) before and after treatment [24].

Determination of bacterial count (CFU)

Under aseptic condition swabs from wounds were taken. These swabs were incubated at 37°C for 24 in nutrient broth. Then cultured, in duplicates dilutions on neomycin blood agar using $100\ \mu\text{l}$ of the diluted specimen for each plate [25] was incubated on 5% CO_2 for 24-48 h at 37°C . The bacterial isolates were confirmed microscopically and biochemically as described in the method of [26]. The bacterial colonies were counted as the method described [27].

Total Phenolic Content (TPC)

Phenolic Content was evaluated by Folin-Ciocalteu reagent, based on the method described [28] with some modification [29, 30] to determine total phenolic content (TPC). Mean TPC value of triplicate assays and expressed as milligram of gallic acid equivalent (GAE) in the gram of honey was reported [31].

Total Flavonoid Content (TFC)

The Mean TFC value of triplicate assays and expressed as milligram of rutin equivalent (RE) in the gram of honey was reported [30].

Free Radical Scavenging Activity

The free radical scavenging activity of Saudi Arabia Qatada (*Acacia hamulosa*) honey sample (1,1-diphenyl-2-picrylhydrazyl - DPPH assay) was determine as described by [31].

Statistical analysis

The data were analyzed using statistical analysis system (SAS) program software; copyright (c) 1998 by SAS Institute Inc., Cary, NC, USA. An unpaired t-test was used for comparison and determining the significance level. Differences were considered statistically significant when the probability of type I error was less than 0.05.

Results

The physicochemical properties of Saudi Arabia Qatada (*Acacia hamulosa*) honey used in dressing included specific gravity of 1.435, pH of 3.56 and, glucose level 378mmol/l. The contents of total phenolic and total flavonoid were 32.15 mg GAE/100 g honey and 54.27 mg RE/100 g honey respectively. The TFC/TPC was 1.68 (**Figure 1**). DPPH radical scavenging activity was exhibited values in the Saudi Arabia Qatada honey sample was 127.83 mg/ml (**Figure 1**).

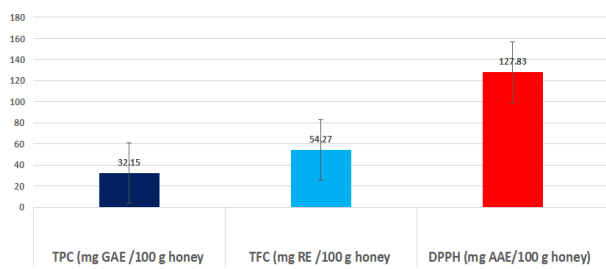


Figure 1. Total phenols, total flavonoids and DPPH in Saudi Arabia Qatada honey sample.

The patients suffering from pilonidal sinus were treated in this study either in Saudi Arabia Qatada dressing honey or standard dressing (normal saline used as a diluent of povidone-iodine solution 10%). The mean duration of 43.5 days (range 31 - 56 days) to be ready for surgical closure was taken in Pilonidal sinus wounds in the standard control dressing group. While a mean duration of 28.5 days (range 21 to 36 days) to achieve similar status required the pilonidal sinus wounds in the honey dressing group. These results revealed a statically significant difference in the duration. Less pain, edema, and foul-smelling discharge were expressed in all patients in the honey group if compared with the standard dressing group (**Figure 2:A-H**).

Total bacterial count during this study revealed that the total bacterial count was 9×10^9 at the begging of the experiment then it reduced after the application of standard dressing traditional therapy to (9×10^5) while Saudi Arabia Qatada honey treatment reached to (9×10^2) after 21 days post-treatment as shown in **Figure 3**. 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 percent of bacterial isolated before treatment were 36% (*Streptococcus* sp.), 26% (*Staphylococcus aureus*), 12% (*Pseudomonas aeruginosa*), 8% (*Escherichia coli*), 7% (*Proteus* sp.), 3% (*Enterococcus* sp.), 2% (*Bacteroides*), 2% (*Acinetobacter*

2%). 2% of isolates were showed mixed infection where 2% has no growth when examined bacterial cultures. In the control conventual antibiotic-treated group there was one patient has no growth where *Enterococcus*, *Pseudomonas*, and mixed organisms remained infected until the end of the study. At the end of the treatment became eventually culture-negative against *Staphylococcus aureus* or *Streptococcus* in the wounds dressing group (**Figure 4**).

The antibiotic sensitivity test was done with the different bacterial strains isolated from the pilonidal sinus wounds. The bacterial strains were not sensitive to Doxycycline, Ciprofloxacin, Ofloxacin, Piperacillin, Cefotaxime, Cefixime, Ampicillin- sulbactam, Cefadroxil, Tazobactam, while moderate effect tigecycline, levofloxacin, and resistance to ampicillin and piperacillin.

Quantification of patient's sera cytokines

Results of honey therapy showed a significant decline in Inflammatory serum cytokines (**Figure 5**). It was clear that in serum a significant ($p < 0.05$) reduction relative to the corresponding pre-treatment values of $\text{TNF}\alpha$ (-52), IL6 - 46), and IL-17 (-60).

Discussion

The physicochemical properties of the Saudi Arabia Qatada (*Acacia hamulosa*) honey were included specific gravity of 1.435, pH of 3.56 and glucose 378mmol/l, [32], contents of total phenolic 32.15 mg GAE/100 g, total flavonoid content 54.27 mg RE/100 g and DPPH radical scavenging activity 127.83 mg/ml (**Figure 3**). Results showed in **Figure 3** indicated that Qatada (*Acacia hamulosa*) a good property and safe for use in the dressing process TPC of the tested Saudi Arabia Qatada honey sample was 32.15 mg GAE/100 g honey. TFC exhibited values in the honey sample 54.27 mg RE/100 g honey. The TFC/TPC was 1.68 (**Figure 3**). DPPH radical scavenging activity was exhibited values in the Saudi Arabia Qatada honey sample was 127.83 mg/ml.

TPC is considered a sensitive, fast and simple method to measure the total phenol in honey [30-34]. Honey is a natural dietary antioxidant [35]. The TPC value in this investigation (32.15 mg GAE/100 g honey) reported for The TPC results were higher than commercial Indian (47-98mg GAE/100 g honey) honey, Germany (4.6mg/100 g honey) honey [36]. Slovenia (4.48mg GAE/100g honey) honey [34], Romania honey (2.00-39.00mg GAE/100 g honey) [39] Argentinean honey (18.730-107.213 mgGAE/100 g honey) [36], from Burkina Faso honey (32.59-114.75mg GAE/100 g honey) [36]. The results of TFC found that the Saudi Arabia Qatada honey sample exhibited higher TFC (**Figure 1**) values (54.27 mg RE/100 g honey). The TFC results in this study were higher than those values reported for Spanish rosemary (0.50-2.00mg/100 g honey) honey, Portuguese heater (0.06-0.50mg/100 g honey) honey [37], and 18.511-32.866 mg RE/100 g acacia honey from Malaysia [38]. The TFC/TPC was 1.68 (**Figure 1**).

The DPPH radical scavenging activity of the Saudi Arabia

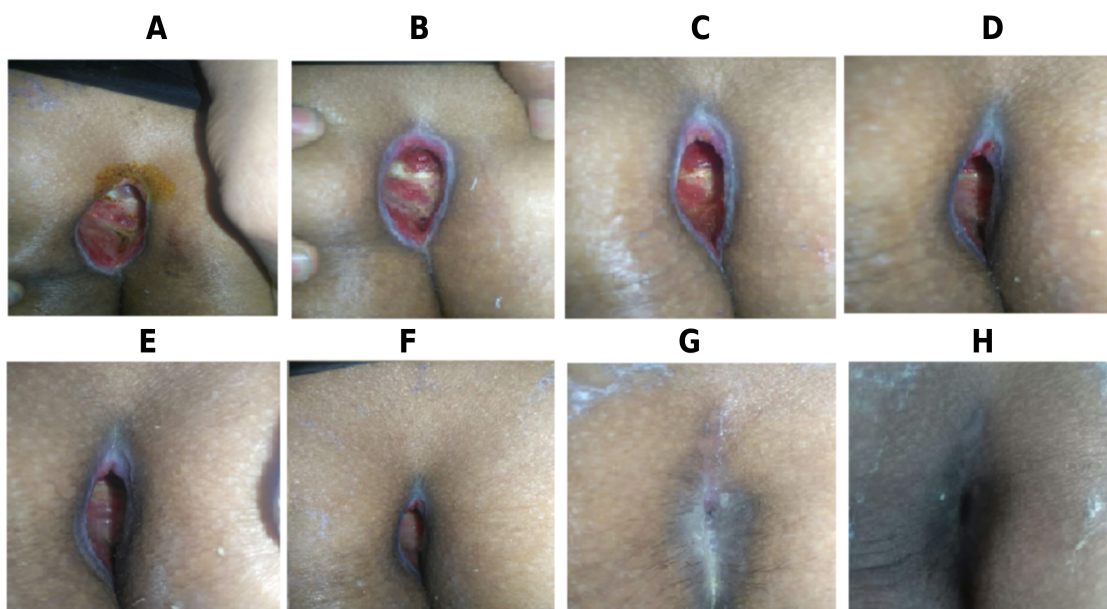


Figure 2. Effect of honey on steps of healing of open method of pilonidal sinus.

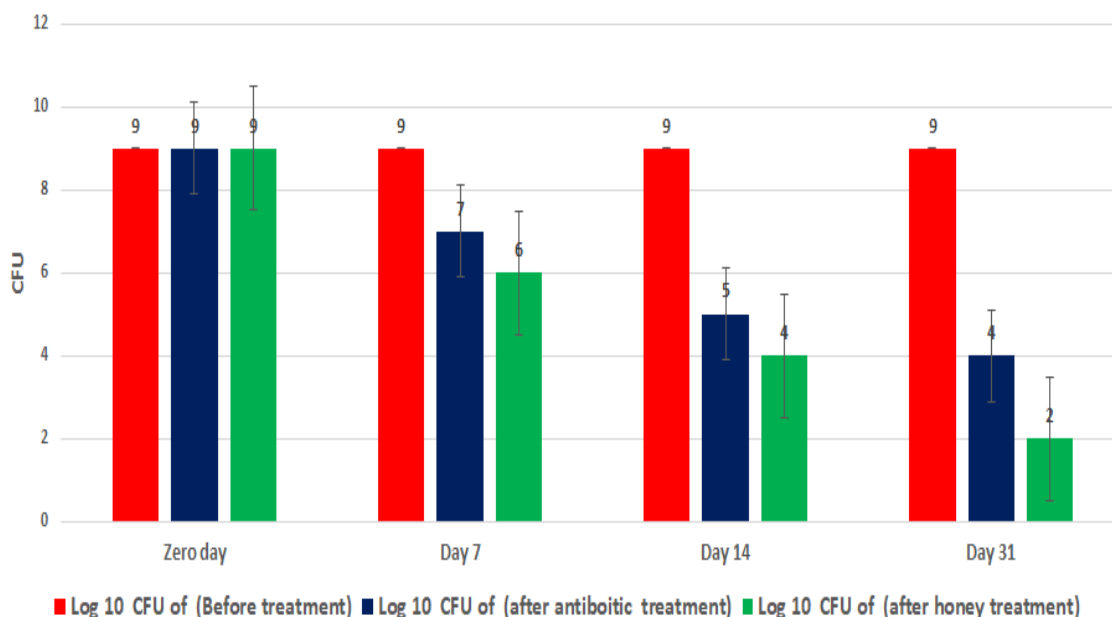


Figure 3. Log10 bacterial CFU and treatment with Saudi Arabia Qatada honey sample.

Qatada honey sample was measured The values of DPPH radical scavenging activity was exhibited in the Saudi Arabia Qatada (*Acacia hamulosa*) honey sample was 127.83 mg/ml. From **Figure 1** indicates that Acacia honey has the highest scavenging activity [39].

Honey has antibacterial, antioxidant activities as well as honey does not have any toxicity to human tissues [40] leading to honey has not antibiotics resistance to be allowed. So, the uses of honey

as a medicine have been rediscovered both in in vitro studies and in clinical trials [41]. Honey has been known as an agent that is able to encourage the repair of wounds, guaranteeing healing with little or no scar formation [12,41].

In this investigation, showed a slightly shorter duration in Saudi Arabia Qatada honey for Pilonidal sinus wound healing (mean of 28.5 days) if compared with the controlled group (mean of 43.5 days). It was clear that the difference was statistically

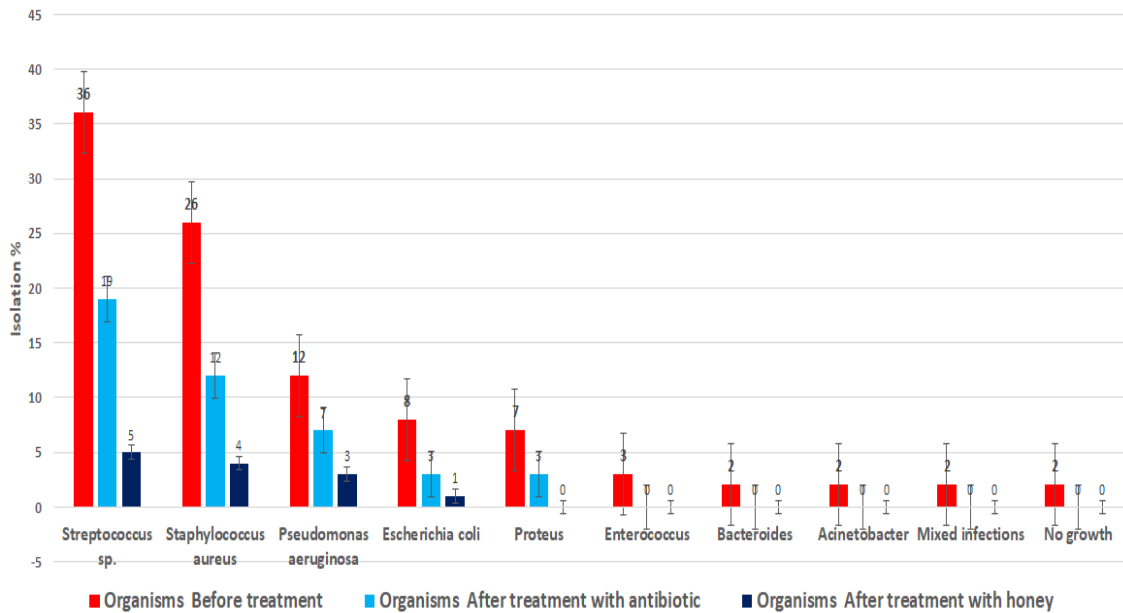


Figure 4. The percentage of isolated bacterial strains before and after treatment.

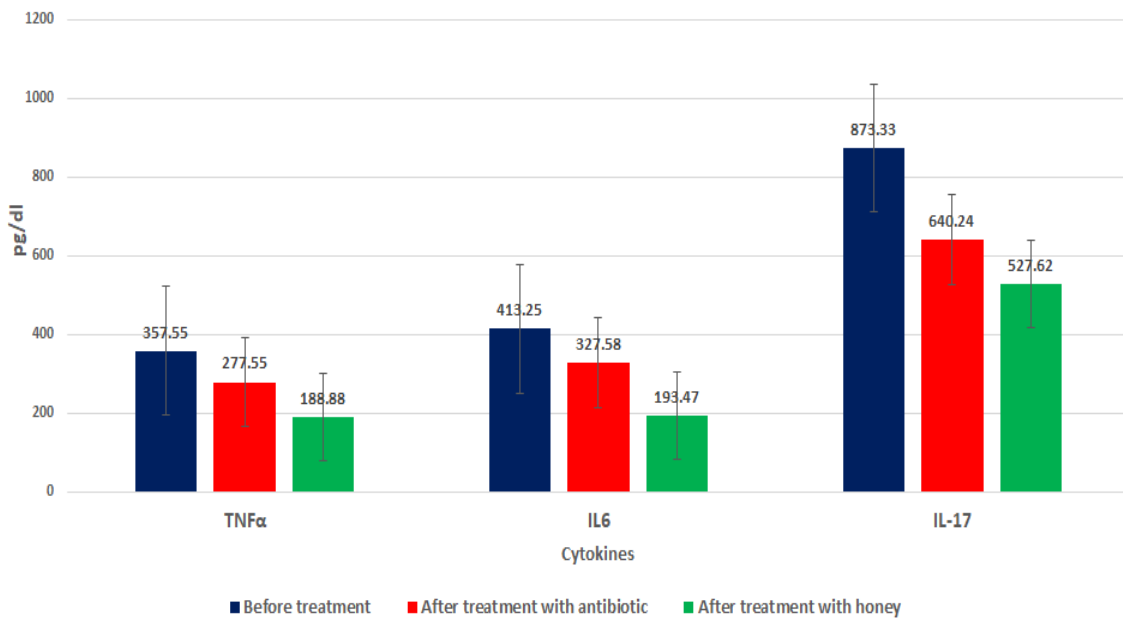


Figure 5. Cytokines measured before and after treatment.

significant. The tissue granulation formation, epithelization and wound contraction were involving. Wounds variable sizes in this study were observed, so the quantitative did not able to measure. While the wounds were clean and ready for closure. No toxic effect of honey in the treatment on the keratinocytes and fibroblasts [42], increase in the regenerative capacity of skin cells [43], promote re-epithelialization [40], increases wound contraction [44], rapid suppression of inflammation [45], rapid clearance of

infections [12] minimization of scarring [46] stimulation of angiogenesis [47] tissue granulation and epithelium growth [48] safe, cost-effective [49] and beneficial dressing biomaterial for wound management [50].

The present investigation showed that the total bacterial count was 9×10^9 at the begging of the study then it reduced after the application of standard dressing traditional therapy to (9×10^5) and Qatada honey (9×10^2) after 21 days post-treatment. This

result proved probably attributed to the significant antibacterial activity of honey [12].

Streptococcus sp. was commonest organism followed by *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The antimicrobial properties of honey related to organisms growth inhibition has been well documented [55]. Also, undiluted honey has been attributed to its high antimicrobial activity [12, 51], inhibin [52] osmotic effect, acidity, hydrogen peroxide. It is clear that computation with honey dressing and systemic antibiotics additively work to eradicate a bacterial infection from the wound. This was evident in the percentage levels of bacterial sensitivity [53]. Also, there no new organisms were found in the wound of both groups, thus the findings indicating that both methods of treatment were able to prevent cross-infection [50,53, 54].

Saudi Arabia Qatada (*Acacia hamulosa*) honey therapy showed an effect on serum, TNF α (-52), IL6 - 46), and IL-17 (-60) relative to the corresponding pre-treatment values. These results showed in the wound healing a similar finding abstained by [55, [56] who found that monocytes and macrophages release proinflammatory cytokines from surrounding cells due to honey treatment in wound which involved in healing as well as exhibits in the skin different immunological functions [57]. Also, many other cytokines and growth factors produced by macrophages that stimulate new capillary growth, fibrosis, collagen synthesis [58]. Activated macrophages inflammatory mediators, including tumor necrosis factor- α (TNF- α), nitric oxide (NO), and IL-6. IL-4. which play an important role in wound healing and angiogenesis [59].

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