Clinical Trial: Bee Honey As Topical Treatment For Infected Chronic Wound

Mahasin wadi[1]*,Talal Geregandi[2], Dr. Hanan Alyami[3]

[1*]Ph.D. Medical Microbiology, Department of Medical-Surgical, College of Nursing, Princess Nourah Bint Abdulrahman University, Riyadh, Saudi Arabia MAWadihassan@pnu.edu.sa
[2]Associate Professor MD, Clinical Surgery, MD Plastic Surgery& Reconstructive Surgery. Khartoum North Teaching Hospital , Surgery & Plastic Surgery Unit. Khartoum , Sudan. t_geregandi@yahoo.com Mobile +249122449190
[3]Ph.D Assistant Professor, Department of Medical and Surgical Nursing, College of Nursing Princess Nourah Bint Abdulrahman University, Riyadh, Saudi Arabia. HMALYMI@pnu.edu.sa

***Corresponding author:** Mahasin Wadi
*Assistant professor, Ph.D. Medical Microbiology, Department of Medical Surgical, College of Nursing Princess Nourah Bint Abdulrahman University, Riyadh, Saudi Arabia. MAWadihassan@pnu.edu.sa - Mobile +966502192600

ABSTRACT
Background: Many studies have demonstrated that honey has antibacterial activity *in- vitro*. Honey was proved clinically to be effective to severe infected chronic wounds not responding to conventional dressing.
Objective: To evaluate the effect of topical application of honey to chronic wound with infected necrotic tissue.
Methods: A female 48 year old was admitted to Khartoum North Teaching Hospital, with open fracture of tibia and fibula with chronic infected ulcerated wound. Swabs were taken from the infected wound for isolation and identification and viable bacterial count of the causative organism. Primary care of the wound was done which required grafting with split-thickness skin grafts and dressing with MEBO ointment was used. Daily topical application of honey was used instead of MEBO ointment
Results: Isolated organism has been identified as *Staphylococcus aureus*, an *In vitro* antibacterial test of honey showed significant activity against the isolated organism 28mm inhibition zone. Daily topical application of honey resulted in clean healthy granulation tissue after 2 weeks of treatment. Wound was reduced in size with healthy granulation tissue and split skin graft was done and complete healing was obtained after 6 weeks.
Conclusion: Honey accelerates wound healing with promotion of healthy granulation tissue in short period. Honey exerted strong antibacterial activity against infected organism and helped graft taking.

Key words: Bee Honey, Topical application Treatment, Infected Chronic Wound.

INTRODUCTION

Unhealed wounds, ulcers, and burns have a significant negative impact on both public health and the economy. In both humans and animals, wounds are common and can be difficult to treat, especially when they don't heal or get secondary infections from bacteria that are resistant to multiple antibiotics. [1]. Traditional approaches to wound care require the extensive cleaning of the wound with sterile solutions to reduce microbial burden, debridement of any obvious necrotic or contaminated tissues, and the use of the proper wound dressings [1]. Wounds are accompanied by pain and social and emotional damage, both of which can significantly lower quality of life [2-3]. Chronic non-healing wounds are associated with venous and arterial diseases, diabetes, neoplasia, immobility and age [4]. Diverse cell types, secreted growth factors, cytokines, the extracellular matrix, and diverse enzymes all play a role in the healing of a wound. The process of wound healing involves a variety of cells, including platelets, neutrophils, monocytes, macrophages, fibroblasts, keratinocytes, endothelial cells, epithelial cells, and myofibroblasts. Fibroblasts are among all cells and have long been known to be important for wound healing [5].

Fractures of the tibia can be fixed both externally as well as internally but external fixation is a preferred way of treatment in open tibia fracture [6]. Even most of unstable closed shaft fractures can be treated with method of the external fixation in a more efficient manner than with other methods. Due to its subcutaneous localization is 10 suitable for the application of the external fixator [7].

Easy applicability and minimal hinderence with the blood supply of the tibia has made external fixation a very popular mode of treatment, but these advantages have been out weighed by the high incidence of pin-track infection, difficulties relating to soft-tissue management and the potential for malunion. Open fractures of the tibia shaft remain to be one of the most complex, problematic and 3 controversial orthopedic injuries [8]. The other causes being are motor vehicle accident, fall from height, fall of a heavy 4 object [9]. A reasonable therapeutic approach with a low incidence of soft tissue problems appears to be the preservation of temporary external fixation following open reduction and internal fixation ORIF synthesis for the duration of the initial stage of bone healing [10].

Honey decreases inflammatory edema, attracts macrophages, accelerates sloughing of devitalized tissue, provides a local cellular energy source, forms a protective protein layer over the wound and encourages the development of a healthy

granulation bed [11] .Honey has an antibacterial property, which is attributed to its high osmolarity and acidity (pH 3.6–3.7). It also contains antioxidants and encourages angiogenesis and fibroblast growth through the presence of low levels of hydrogen peroxide [12-13]. Honey arrests bacterial cell division, decreases wound pH, hinders biofilm formation, and within 24 hours of honey application odor can be reduced or eradicated. It also has anti-inflammatory properties which decrease oedema and exudate[14]. Honey not only reduces odor through reductions in various bacterial loads but also through providing glucose as a form of energy, which microorganisms use in preference to amino acids from which the malodorous substances are produced, unlike metronidazole, which only targets odor from costs increase for lesions with long healing times or for larger ulcerations, as well as for ulcers that are defined as difficult to treat [14]. Difficult to treat cases can cause significant morbidity [15]. Seriously impact the patient's quality of life and consequently increase treatment costs evidence [16].The honey dressing material sped up the palatal wound's healing process [17]. Honey Usefulness and efficacy of honey dressing in sickle cell patients' chronic unresolved leg ulcers [18]. After children had their teeth extracted, honey reduced the size of the wound and enhanced the healing process [19] Chronic scalp sores can be successfully treated using honey and a silver ion dressing [20]. Due to its antibacterial and immunomodulatory properties, honey helps with the procedures of wound healing [21]. A polyfloral honey from Slovakia that was produced sustainably showed excellent *in vitro* antibacterial effects against bacteria that frequently colonize and infect chronic wounds [22]. Results showed that honey is an effective dressing agent instead of conventional dressings, in treating patients of diabetic foot ulcer [23]. manuka honey has been used as a wound dressing and demonstrated its ability to accelerate autolytic debridement, reduce inflammation, and promote the formation of tissues for wound repair [24]. When compared to the negative control, honey film is more effective at accelerating the healing of burns and incision wounds [25]. At 20% antibacterial honey concentrations, all *P. aeruginosa* strains, including both resistant types and sensitive ones, were suppressed in vitro[26] .According to clinical trials, using honey to treat wounds accelerates up wound healing and lowers the risk of infection when compared to more traditional methods [27].

The tested honeys from Kazakhstan showed an antibacterial effect against the skin- and wound-infecting microbes. Potential bioactive agents for the treatment of wound and skin infections could be substances identified as correlating with antimicrobial activity [28]. It has been shown that the topical use of honey accelerates up the healing of surgical wounds that are severely infected. Additionally, it has aided in the healing of wounds that were infected with pathogens that were resistant to standard treatments like antibiotics and antiseptics, including methicillin-resistant *Staphylococcus aureus* [29]. Phenolic chemicals, flavonoids, methylglyoxal, and Bee defencin are certain compounds that can be found in honey that have antibacterial effects[30]. Following a period of three weeks of topically applying honey dressing to a lesion with hypergranulating tissue, the wound was clean, sterile, and had good granulation tissue. A fast graft was also seen. Without the use of surgery, the hypergranulation tissue was reduced and the incision completely healed [31].

The aim of the current prospective study is to evaluate the healing effect of topical treatment of bee honey on the infected chronic wound.

Ethical Approval

Ethical approval was obtained from the Ethics Committee Khartoum North Teaching Hospital Surgery and Plastic surgery Unit. Khartoum ,Sudan

MATERIALS and METHODS

Honey Sample

A natural, unprocessed, sterile honey sample obtained from an apiary (Sudan), was applied topically to an infected chronic wound. Honey sample was kept in sterilized glass jar was stored at room temperature. The sample was labeled according to the source, location, pH, and date of collection,. At the microbiology laboratory, a honey sample was examined for sterility using standard microbiological techniques.

Clinical Isolates

Sterile swabs were utilized to collect clinical specimens from chronic wound before, during, and after applying wound dressing, for identification, viable count, and sensitivity testing.

In vitro Antibacterial Activity of Bee Honey

Inoculum Preparation

Susceptibility to antibacterial agents was maintained using pure culture and a conventional inoculum size. The test and control organisms were suspended in sterile saline to match the optical density of 1.5 X 108 colony forming units (CFU/ml) provided by the 0.5 McFarland standard tube, which is commercially available. Due to honey's high viscosity, the well plate technique was utilized to test its antibacterial effectiveness.

Well Plate Technique

Muller Hinton agar was reconstituted, sterilized (using an autoclave) at 121°C for 15 minutes, allowed to cool at 48°C, and inoculated with 0.1 ml of standardized 24 broth culture of bacterial suspensions that matched the turbidity of the 0.5 McFarland standard tube (1.5X108) (FU/ml). For assessing the antimicrobial susceptibility of the isolated organisms. standard antimicrobial test was adopted the National Committee for Clinical Laboratory Standards'. 20 ml of the seeded medium was aseptically set into 95 mm-diameter sterile Petri plates before being left to solidify. The inoculated agar plate that had solidified was then kept at 4°C. Using an 8 mm sterile cork borer, four wells (8 mm in diameter) were made, and

the agar cut-disc was removed. A small amount of honey was carefully added—0.2 ml. For 18 to 24 hours, inoculated plates were incubated at 37°C. The diameter of the inhibition zone was expressed in millimeters (mm). Each organism was tested against four replicates of the honey sample, and the results were expressed as means [31].

Patients

Female, 48 year old was admitted to Khartoum North Teaching Hospital, surgery and plastic surgery unit with open fracture of the right distal tibia and fibula with expose bone during December (2021).Duration of the wound was 3 months. The patient was admitted with open fracture with infected wound involved anterior, posterior medial and lateral aspects of the distal right lower limb due to severe trauma. She was referred from the orthopedic clinic, Khartoum North Teaching Hospital. The patient had been treated throughout this period by conventional methods of dressing (saline and MIBO ointment) and antibiotics with poor response and recurrent wound infection and failure of graft taking. Swabs were taken from the infected wound for isolation and identification and viable count of the causative organism. On admission to the Hospital the wound was infected with full of pus and necrotic tissue. Surgical dressing and debridement of the wound was done under general anesthesia followed by daily dressing of honey. During an initial round of treatment, weekly culture swabs were collected. Primary treatment of the wound was done and the fracture was stabilized with external fixation due to the large soft tissue destruction Figure (A-1, A-2 , A-3, A-4). The patient was admitted with sign of infection; pain , edema, malodor and necrotic tissue with high exudate and slough. The wounds were observed for evidence of epithelialization, granulation tissue, and rate of wound contraction. The wound was largely opened with exposed bone. The patient is non-diabetic and non-hypertensive. The first debridement was done for removal of dead tissue and bone. Daily dressing of the wound with saline and topical application of MEBO ointment (combination of honey and herbs), was carried out. The patient was on Cetrixone (Samixon) intra venous / 1 Gram twice daily. Metronidazole 500 µg infusion every 8 hours was given . She received 4 units of blood.

Wound graft and flab was carried out to cover the ulcerated part.

Swabs were taken from the infected wound for isolation and identification of the causative organism. Dressing was changed instead of saline and MEBO. Daily topically application of honey to the Infected wound was carried out. Wound was photographed before and during honey dressing.

The patients' age, general and disease-related medical history wound history, and wound aetiology were monitored prior to the first application of honey dressings. A full wound assessment was conducted at various points in the study. This involved determining the wound surface area (in cm2), depth (measured by filling the wound up with sterile isotonic sodium chloride solution), and wound cleanliness, i.e. absence of slough/necrotic tissue. In addition, all patients were asked to grade their perceived level of pain.

Natural sterile honey sample supplied from the apiary was used for topical application of infected wound.

RESULTS

A 48 year old female was admitted to Khartoum North Teaching Hospital, Surgery and plastic surgery unit with open fracture and infected wound due to severe trauma. *Staphylococcus aureus* has been identified as an isolated organism using the conventional microbiological assays. Honey sample tested for antibacterial sterility showed no bacterial growth. *In vitro* antibacterial test of honey showed significant activity against the isolated organism 28mm inhibition zone. Bacterial count before honey application, showed high numbers of bacteria. Primary treatment of the wound was done tibia and fibula fracture was stabilized by external fixation. Debridement of dead tissue was done under general anesthesia. Dressing with normal saline and topical application of MEBO ointment twice a week was carried out. Recurrent infection , contracture of graft and flab was observed .

Swabs has been taken from the wound for microbiological identification revealed high infection with *Staphylococcus aureus* .

Daily topical application of bee honey on infected wound resulted in clean wound without malodor after 3 days (Figure B-3). Bacterial count was reduced in number after 3 days.

After 7 days of topical application of honey to the infected wound a decrease in the amount of necrosis and slough was observed as well as increase in the percentage of granulation and epithelial tissue Figure (B-5).

Swabs from the wound were subjected to bacterial count showed no bacterial growth and clean wound free of bacteria was obtained after 10 days of topical honey dressing. Healthy granulation tissue was obtained and the surrounding skin improved and wound size was decrease Figure (B-6).

After 2 weeks of honey dressing , wound was decrease in size and showed healthy granulation tissue, Figure (B-7&8). Clean wound with healthy granulation tissue after 4 weeks of honey application , split skin graft was performed Figure (B- 9& 10). After 6 weeks of honey dressing healing was achieved and wound closure was obtained in short period.

DISCUSSION

The potential of medical-grade honey dressings to encourage autolytic debridement of devitalized tissue within wounds has been demonstrated to be favorable, greatly lowering malodor and pain.

Unhealed wounds has a great impact on public health. Non healing wound with secondary infection resulted in chronic wound with recurrent infection. these slow- or non-healing wounds pose a significant risk of sepsis and can result in invasive inflammatory disease such as infective endocarditis, which is associated with high mortality and morbidity rate [17] .

A 48 year old female admitted to the Khartoum North Teaching Hospital with opened fracture of the right tibia and fibula,

with large destructive tissue and infected wound with necrotic tissue and offensive odor. Debridement of dead tissue was done under general anesthesia. Dressing with normal saline and topical application of MEBO ointment twice a week was carried out. Lack of response to the topical application of MEBO ointment resulted in recurrent infection. Daily bee honey application instead of MEBO ointment showed decrease in necrotic tissue, slough, and disappearance of malodor. Swabs showed reduction in bacterial count, and appearance of healthy granulation tissue was observed. Wound size was reduced and the percentage of healthy granulation tissue was increase and sterile wound was obtained. Honey has known as broad spectrum antibacterial effect [31], which could have contribution to the accelerate wound healing [12]. The application of honey-impregnated gauze keeps the wound moist, protects against infection, and stimulates quick epithelialization. After one week of honey treatment all swabs were void of bacteria and wound healing was noted with healthy granulation tissue. Prompt graft taking was reported. Honey decreases inflammatory oedema, attracts macrophages, accelerates sloughing of devitalized tissue, provides a local cellular -energy source, forms a protective protein layer over the wound and encourages the development of a healthy granulation bed [14].

The findings of the current study showed that after 4 weeks of honey treatment healing and skin graft taking were obtained. Our findings supported the previous study that confirmed the use of honey on chronic wounds might reduce healing time compared with some conventional dressing [18]. The rapid clearance of infection is the most notable feature of honey because it is effective against aerobic, anaerobic Gram - positive, Gram - negative bacteria, and a variety of fungi[19]. The antibacterial activity of honey is partly due to its hygroscopic properties, acidic pH, hydrogen peroxide content and phytochemical factors [20].The discovery of hydrogen peroxide (H_2O_2) as an intrinsic compound of honey brought an assumption that (H_2O_2), is a main factor underlying the broad antimicrobial activity of honey [21].

The findings of the present showed that honey was an effective and feasible treatment of non-healed wound Using honey as a wound care product has been recognized because it is believed to positively influence the wound healing process. The viscosity, water content, sugars (mainly glucose and fructose), antioxidants, a variety of amino acids, vitamins, and minerals, glucose oxidase which generates hydrogen peroxide and supplies the majority of the antibacterial activity and gluconic acid which gives honey an acidic pH of 3.2 to 4.5 are the elements and characteristics of honey that are relevant to wound healing [32]. Today there are several medical grade honey-based dressings that are approved by the United States Food and Drug Administration (FDA). Various mechanisms have made honey superior to many other available medically-approved wound care products. Honey has a hygroscopic effect by attracting and holding excessive fluid from the surrounding environment and thus reduces inflammatory oedema and exudation associated with the healing process [22]. (Simon et al., 2009; Al-Waili et al. The study's findings demonstrated the safety and effectiveness of using the tried-and-true honey to treat wounds. These outcomes were consistent with previous research that used honey's anti-microbial and anti-fungal properties to treat wounds[22].

The present study showed that sloughy, necrotic wounds were cleaned effectively with honey dressings, reflecting observations from previous studies [31]. Due to its high sugar content, ability to produce reactive oxygen species, and anti-inflammatory qualities, honey may help regenerate damaged tissues and promote wound healing [33]. Honey can also prevent wound infections, promote tissue growth and re-epithelization, and lessen the creation of scars. In order to combat bacteria that are resistant to several drugs, scientists have focused on natural compounds that have minimal toxicity and antibacterial and antioxidant properties.

Generally wounds healed well after application of honey products, reflecting the results of many surveys (16) as well as other studies that have been published. Approximately 85 % of the wounds healed or improved during the relatively short period of investigation. Thus, the presence of an active healing component in the honey products used is strongly suggested as also indicated in a randomized clinical trial (10).

Conclusion

Concentrating on the antibacterial capabilities of natural compounds, which can help with wound healing, as resistance rises.

Honey dressings have been proven to be a dependable and affordable option, especially in underdeveloped nations. Topical honey-based wound dressings efficiently control and treat a range of wounds, such as pressure ulcers, diabetic ulcers, surgical wounds, and burns. Honey ensured infection control, sped up the healing process, and was reasonably priced. Honey also accelerated wound contraction and epithelialization during the healing process.

Acknowledgements

The authors express their gratitude to Princess Nourah bint Abdulrahman University Researches Supporting Projects number (PNURSP2024R 386), Riyadh, Saudi Arabia.

Availability of data and materials

All data and materials were included in the manuscript.

Funding

This study was supported by Princess Nourah bint AbdulrahmanUniversity Researches Supporting Projects number (PNURSP2024R 386), Riyadh, Saudi Arabia.

Ethics approval and consent to participate

Ethical approval and patient consent was obtained from the Ethics Committee Khartoum North Teaching Hospital Surgery

and Plastic surgery Unit. Khartoum ,Sudan.

Competing interests
Not applicable

REFERNCES

1. Tosic A, Ebraheim NA, Abou Chakra I, Emara K. Tosic external fixator in the management of proximal tibial fractures in adults. Or thopedics 2001;24:581-4
2. Dougher ty PJ, Vaidya R, Silver ton CD, Bar tlett C, Najibi S. Joint and long-bone gunshot injuries. J Bone Joint Surg 2009;91:980-97.
3. Atiyeh BS, Dibo SA, Hayek, SN (2009) Wound cleansing, topical antiseptics and wound healing. Inter Wound J 6: 420-430.
4. Phillips T, Stanton B, Provan A, Lew R. A study of the impact of leg ulcers on quality of life: financial, social, and psycho- logic implications. j Am Acad Dermatol 1994; 31: 49–53.
5. Monika P, Waiker PV, Chandraprabha MN, Rangarajan A, Murthy KNC. Myofibroblast progeny in wound biology and wound healing studies. Wound Repair Regen. (2021) 29:531–47. 10.1111/wrr.12937 [PubMed] [CrossRef] [Google Scholar]
6. Ebbeskog B, Ekman SL. Elderly persons' experiences of living with venous leg ulcer: living in a dialectal relation-ship between freedom and imprisonment. Scand j Caring Sci 2001; 15: 235–243.
7. Simon DA, Dix FP, McCollum CN. Management of venous leg ulcers. BMj 2004; 328: 1358–1362.
8. Emami A, Mjoberg B, Karlstrom G, Larsson S. Treatment of closed tibial shaft fractures with unilateral external fixation. Injury 1995; 26(5): 299- 303.
9. Oh CW, Park BC, Kyung HS, Kim SJ, Kim HS, Lee SM, Ihn JC. Percutaneous plating for unstable tibial fractures. J Orthop Sci 2003; 8(2): 166-9.
10. Lavini F, Dall'Oca C, Mezzari S, Maluta T, Luminari E, Perusi F, Vecchini E, Magnan B. Temporary bridging external fixation in distal tibial fracture. Injury. 2014 Dec;45 Suppl 6:S58-63. doi: 10.1016/j.injury.2014.10.025. Epub 2014 Oct 31. PMID: 25457321.
11. Margolis Dj, Bilker W, Knauss j, Baumgarten M, Strom BL.The incidence and prevalence of pressure ulcers among elderly patients in general medical practice. Ann Epidemiol 2002; 12: 321–325.
12. Mathews HA, Binnington AG (2002) Wound managment using honey. Compend Contin Educ Vet 24: 53–60
13. Seckam A & Cooper R. Understanding how honey impacts on wounds: an update on recent research findings. Wounds Int 2013; 4(l):20-24.
14. Molan P. The evidence and the rationale for the use of honey as a wound dressing. Wound Practice and Research 2011; 19(4):204-219. Retrieved 2012.
15. Belcher I. A review of medical-grade honey in wound care. Br J Nurs 2012; 21(15):S4-S9.
16. González-Consuegra RV, Verdú J (2011). Quality of life in people with venous leg ulcers: an integrative review. J Adv Nurs. 67(5):926-44. doi: 10.1111/j.1365- 2648.2010.05568.x
17. Alasqah M; Alrashidi A; Alshammari N; Alshehri A; Gufran K, European review for medical and pharmacological sciences [Eur Rev Med Pharmacol Sci], Effect of honey dressing material on palatal wound healing after harvesting a free gingival graft: a prospective randomized case control study ISSN: 2284-0729, 2022 Apr; Vol. 26 (8), pp. 2662-2668
18. Nwagu, M. U., & Omokhua, G. I. (2020). Treatment of recalcitrant chronic leg ulcer in a known sickle cell anaemia patient using honey and fresh hbaa red cell concentrate in a Nigerian secondary healthcare facility. Annals of African Medicine, 19(4), 278–281. https://doi-org.sdl.idm.oclc.org/10.4103/aam.aam_53_19
19. Mokhtari, S., Sanati, I., Abdolahy, S., & Hosseini, Z. (2019). Evaluation of the effect of honey on the healing of tooth extraction wounds in 4- to 9-year-old children. Nigerian Journal of Clinical Practice, 22(10), 1328–1334. https://doi-org.sdl.idm.oclc.org/10.4103/njcp.njcp_102_19
20. Lin, J.-Q., Yang, L.-L., Yang, X., Zeng, X.-H., Zhang, Y., Li, Z.-P., & Liu, H.-Y. (2022). Honey combined with silver ion dressing in complicated chronic wound of the scalp: a case report. European Review for Medical and Pharmacological Sciences, 26(14), 5245–5249. https://doi-org.sdl.idm.oclc.org/10.26355/eurrev_202207_29314
21. 21. Bulut, S. P., Gurbuzel, M., Karabela, S. N., Pence, H. H., Aksaray, S., & Topal, U. (2021). The investigation of biochemical and microbiological properties of four different honey types produced in turkey and the comparison of their effects with silver sulfadiazine on wound healing in a rat model of burn injury. Nigerian Journal of Clinical Practice, 24(11), 1694–1705. https://doi-org.sdl.idm.oclc.org/10.4103/njcp.njcp_582_20
22. Zemanova, M., Slobodnikova, L., Cambal, M., & Labas, P. (2021). Excellent antibacterial activity of Slovak honeys on bacteria mostly infecting chronic wounds. Bratislavske Lekarske Listy, 122(7), 519–525. https://doi-org.sdl.idm.oclc.org/10.4149/BLL_2021_084
23. Imran, M., Hussain, M. B., & Baig, M. (2015). A Randomized, Controlled Clinical Trial of Honey-Impregnated Dressing for Treating Diabetic Foot Ulcer. Journal of the College of Physicians and Surgeons--Pakistan : JCPSP, 25(10), 721–725. https://doi-org.sdl.idm.oclc.org/10.2015/JCPSP.721725
24. Kapoor, N., & Yadav, R. (2021). Manuka honey: A promising wound dressing material for the chronic nonhealing discharging wounds: A retrospective study. National Journal of Maxillofacial Surgery, 12(2), 233–237. https://doi-org.sdl.idm.oclc.org/10.4103/njms.NJMS_154_20

25. Febriyenti, F., Lucida, H., Almahdy, A., Alfikriyah, I., & Hanif, M. (2019). Wound-Healing Effect of Honey Gel and Film. Journal of Pharmacy & Bioallied Sciences, 11(2), 176–180. https://doi-org.sdl.idm.oclc.org/10.4103/jpbs.JPBS_184_18
26. Shenoy, V. P., Ballal, M., Shivananda, P., & Bairy, I. (2012). Honey as an antimicrobial agent against pseudomonas aeruginosa isolated from infected wounds. Journal of Global Infectious Diseases, 4(2), 102–105. https://doi-org.sdl.idm.oclc.org/10.4103/0974-777X.96770
27. Gethin G, Cowman S. 2008. Manuka honey vs.hydrogel – a prospective, open label, multicentre, randomised controlled trial to compare desloughing efficacy and healing outcomes in venous ulcers. J Clin Nurs 18: 466–474.
28. McLoone, P., Zhumbayeva, A., Yunussova, S., Kaliyev, Y., Yevstafeva, L., Verrall, S., Sungurtas, J., Austin, C., Allwood, J. W., & McDougall, G. J. (2021). Identification of components in Kazakhstan honeys that correlate with antimicrobial activity against wound and skin infecting microorganisms. BMC Complementary Medicine and Therapies, 21(1), 300. https://doi-org.sdl.idm.oclc.org/10.1186/s12906-021-03466-0
29. Haynes JS, Callaghan R. Properties of honey: Its mode of action and clinical outcomes. Wounds UK 2011;7:50-7.
30. Molan P, Rhodes T. Honey: a biologic wound dressing. Wounds. 2015;27(6):141-151
31. Wadi, M., & Geregandi, T. (2020). Efficacy of Bee Honey on Wound Healing: Split Skin Graft with Hyper-granulation Tissue. Journal of Natural Remedies, 20(2), 71–78. https://doi.org/10.18311/jnr/2020/24172
32. Jull AB, Rodgers A, Walker N (2008) Honey as a topical treatment for wounds. Cochrane Database of Systematic Reviews 4: CD005083
33. Mieles, J.Y.; Vyas, C.; Aslan, E.; Humphreys, G.; Diver, C.; Bartolo, P. Honey: An Advanced Antimicrobial and Wound Healing Biomaterial for Tissue Engineering Applications. Pharmaceutics 2022, 14, 1663. [CrossRef

Figure A -1 Open fracture of the right distal tibia and fibula with external fixation, heavy infection before honey dressing.

Figure A-2 Open fracture with infected necrotic wound before honey dressing

Figure B-3 Open chronic wound after 5 days of honey application

Figure B-4 Open chronic wound after 5 days honey application.

Figure B-5 Open chronic wound after 7 days of honey application.

Figure B- 6 After 10 days of honey application

Figure B-7 Clean wound with healthy granulation tissue, reduced in size, after 2 weeks of honey dressing. Application of honey resulted in healthy granulation tissue.

Figure B-8 Clean wound with healthy granulation tissue -reduced in size after 2 weeks of honey dressing.

Figure B-9 Clean wound with healthy granulation tissue after 4 weeks of honey application.

Figure B-10 The wound was grafted by split -skin graft. Healthy granulation tissue was obtained after 4 weeks of honey application. Cleanliness of the wound after honey dressing promoted skin grafting.