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Honey as a topical antibacterial agent for treatment of superficial infected wounds

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Abstract

The excessive use of antibiotics has led to the ever increasing emergence of resistant strains. Honey as a natural source possessing antibacterial activity could be a good alternative to antibiotics. It is a cost effective natural remedy, easily available and has longer shelf life. It is a single product having antibacterial, anti-inflammatory, antiseptic and analgesic properties, all in one. This study aims to find an efficacious alternative to antibiotics used for the treatment of infected wounds which would be much cost effective and do not result in development of resistant organisms. A randomized controlled trial for healing of infected acute partial thickness skin wounds was conducted on 196 subjects treated with Honey in the test group and Triple Antibiotic Ointment with Pain Reliever in the control group. The difference between the baseline parameters among the two groups was statistically insignificant. Bates-Jensen Wound Assessment Tool was used for the assessment of wound healing. The level of the significance (α) was set to be 0.05. Results with p-value less than 0.05 were considered statistically significant. Rapid healing time was noted for honey as compared to the Triple Antibiotic Ointment with Pain Reliever. The mean healing time was 14.06 days for honey while 15.65 days for Triple Antibiotic Ointment. There was an appreciable difference in the reduction of size among the test group at day 5 (p-value of 0.028) and at day 10 (p-value of 0.007). This research demonstrates that honey could be an effective alternative for the treatment of infected skin wounds.

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Introduction

Merriam-Webster defines Wound as "an injury to the body (as from violence, accident, or surgery) that typically involves laceration or breaking of a membrane (such as the skin) and usually damage to underlying tissues." When there is a break in the continuity of the skin and the skin barrier is broken, then the pathogenic organisms invade the viable tissue and hence, the wound become infected, which renders in delaying of wound healing. Mostly the causative agents are the bacteria which are the part of the normal flora. One great example is *Staphylococcus aureus* which is the part of normal flora and also one of the most common agents causing wound infection. Infected wounds remains a challenge for the health care providers, results in delayed healing, increasing patient's morbidity and anxiety as well as huge increase in the health care cost due to the purchase of antibiotics and also the prolonged hospital stay.

After the discovery of Penicillin, antibiotics were longed use for the treatment of infected wound and were considered as the standard of treatment. But in the modern era, the excessive use of antibiotics has lead microorganisms to develop resistance and the ever increasing emergence of resistant strains like Methicillin Resistant *Staphylococcus aureus* (MRSA) and Vancomycin Resistant *Enterococcus* (VRE).

This has created tremendous clinical issues in the treatment of infected wounds which signifies the importance of developing an alternative medicine to the conventional antibiotics for the treatment of infected wounds. World Health Organization (WHO) has also recognized the role of Unani Medicine and encourages its use because of its efficacy, wide availability, fewer side effects and cost effectiveness.

Honey is one of the most widely used alternatives in the Unani System of Medicine for the treatment of infected wounds. Honey causes the production of cytokines, TNF-α, interleulin-1β, interleukin-6, matrix metallopeptidase-9, and many others, together with increase in the activity of plasmin, contributes to the tissue repair and re-epithelization, resulting in the wound healing. The high osmolarity of honey contributes to negative pressure wound therapy and its acidity protects the wounds from destructive effects of proteases. The antibacterial activity of honey is so great that it remains potent even when the honey is heavily diluted by the wound exudate, and is very sensitive to MRSA and VRE. Honey provides a soothing effect, lessen the edema, exudate and foul odor, and also minimizes the wound scarring and prevent eschar formation. Role of honey is well established in debridement of wounds.

Antibacterial activity is also related to the flavonoids present in honey, which are phenolic compounds and are bioactive. Bioactive substances are those which have specific effect or reaction in living organism upon exposure to a substance. Of these flavonoids, pinocembrin is well-recognized in honey. Honey also contains numerous minerals, vitamins and unidentified compound which help in wound healing.

I. Koop has justly said that "bee venom, which is easily obtained, deserves no less attention of the medical profession than antibiotics of fungal and bacterial origin".

This study aims to compare the efficacy of Honey & Triple Antibiotic Ointment with Pain Reliever (Bacitracin, Polymyxin, Neomycin and Lidocaine) as Topical agents for the treatment of infected wounds. Also to identify different types of organism isolated from wounds & to determine the effects of honey on them. Hence the promotion of the use of natural products in clinical practice for wound healing and to provide evidence based controlled trial for the treatment of infected wounds.

Materials and methods

Study Design

A randomized controlled trial was conducted for the treatment of skin wounds. The subjects in the test group were treated with honey and the subjects in the control group were treated with Triple Antibiotic Ointment with Pain Reliever. Single blinding

technique was used for the patients. Randomization was done by the "chit method" and allocation concealment was achieved with sequential numbered envelopes which were sealed and opaque.

Study Setting

The study set up was located at Shifa-ul-Mulk Memorial Hospital, Faculty of Eastern Medicine, Hamdard University at Madinat-ul-Hikmah, Sharah-e-Madinat-ul-Hikmah, Muhammad Bin Qasim Avenue, Karachi. It is a 50 bedded hospital and belong to Private sector. The hospital is mainly welfare in nature and is established for the promotion of Unani Medicine.

Sample size

Sample size was calculated to be 196 using the general formula, with equal number of subjects in each group i.e. 98. Level of significance (α) was set to be 0.05 and β was set to be 0.20 where standardized effect size is 0.40 and standard deviation, 2.

Selection Criteria

The subjects enrolled in Shifa-ul-Mulk Memorial Hospital, were selected on the basis of Inclusion criteria who gave the written informed consent. Inclusion Criteria comprises patients living in the vicinity of hospital within a distance of 50km, belonging to any socioeconomic class, ageing between 6-70 years having superficial wounds involving epidermis and/or dermis, for less than a period of 1 week, who haven't applied any remedy since the development of wound.

While the exclusion criteria comprises patients having wounds with necrotic or granulation tissue or undermining; with damage to muscles, bones or supporting structure; wounds with fibrotic, scarred or hyperkeratotic edges or rolled up margins; wounds with large amount of exudate or having dark red, purple, black, hyperpigmented or non-blanchable skin color surrounding wound; wounds with pitting edema, or non-pitting edema extending more than or equal to 4cm and/or crepitus; peripheral tissue induration extending more than 2 cm around wound. Patients having Diabetes Mellitus, malnutrition or vitamin/mineral deficiencies, hyperpyrexia (>101°F) or any systemic illness or serious chronic disease were also excluded from the study. Lastly, the patients who are having more than one organism detected in culture report, allergic to Honey or Penicillin, the patients on NSAIDs, steroids, immunosuppressive or radiation therapy or the bedridden patients were also excluded.

Data Collection Procedure

Demographic data was recorded and a detailed history and local examination was carried out by the physician. (Table 1 and 2) Swab method was used for the collection of wound specimen and Levine technique was used for swabbing. Venipuncture was used for blood sampling. The wound specimen and blood samples were immediately transferred to the Clinical Laboratory Shifa-ul-Mulk Memorial Hospital, located in the same hospital where main study was conducted.

Table 1. Age distribution.

Age Group	Test group	Control group
6-18	10	7
19-31	26	30
32-44	32	28
45-57	22	21
58-70	8	12
6-70	98	98
Total	196	

Table 2. Sex distribution.

Gender	Test group	Control group
Male	62	59
Female	36	39
Total	98	98

Prepared Blood agar and MacConkey Agar were used as culture media. The samples were immediately inoculated on reaching the laboratory. After incubation the culture plates were examined for microbial growth. Gram staining and specific biochemical test were carried out for proper identification of organisms. Biochemical tests includes oxidase test, catalase test, coagulase test, indole test, triple sugar iron test, hydrogen sulphide test, carbohydrate fermentation test, urease test and citrate utilization test. Cefoxitin disk screen test was used for the detection of MRSA and Methicillin-Sensitive *Staphylococcus Aureus* (MSSA).

Laboratory reports were obtained after 48 hrs. Positive culture was suggestive of wound specimen being infected. If culture report was found to be positive and complete blood count, liver function test, renal function test, uric acid and fasting or random blood sugar are within normal ranges, then the individual undergoes the process of randomization and was allocated in either of the two groups.

According to the allocation of patients in the test or control group, they were treated with the corresponding drug. Sterile gloves were worn, the wound area was cleaned using plain water and the drugs were gently applied on the affected area in long even strokes following the direction of hairs. The quantity used for the drug application was approximately 1 g per cm^2 of the wound area. The drugs were only applied externally. The wounds were daily dressed after application of topical ointment. Bates-Jensen Wound Assessment Tool was used for the assessment of wound on each visit. Each parameter was scored 1-5 and the total was used to access the severity of wound.

The total scores graded the wounds into 4 categories
Scores <13 = Healed wound
Scores 13 – 20 = minimal severity
Scores 21 – 30 = mild severity
Scores 31 – 40 = moderate severity
Scores 41 – 65 = extreme severity

For the measurement of the wound size, acetate sheet was used for accurate measurements. The local and systemic sign of inflammation, due to the application of honey and triple antibiotic ointment, were also assessed as charted in the Performa, along with parameters of Bates-Jensen Wound Assessment Tool. Regional Lymph nodes status was also checked.

Days of each visit were counted until complete healing and the day of first application of honey or triple antibiotic ointment was counted as day 1. The wound culture specimen was again repeated at day 15 and day 30. The patient lost to continue follow up before complete healing, were not evaluated for results.

Test Drug

Honey for the treatment of test group was manufactured from Hamdard Laboratories (Waqf) Pakistan, which was labeled as "Hamdard Honey" and sold in a 120g tube. It was obtained from the bees of wild source from the forests of Peshawar which is located in the Northern Areas of Pakistan. The physical and chemical properties of Hamdard Honey are listed below. The Standard Values are obtained from the Pakistan Standards and Quality Control Authority (PSQCA) (Table 3).

Table 3. The physical and chemical properties of test drug (Hamdard honey).

Parameter	Sample	Standard
Appearance	Thick syrup	Thick syrup
Color	Golden Yellow	Golden Yellow
Odor	Characteristic	Characteristic
Quality/Condition	Satisfactory	Satisfactory
Starch/Dextrin	Negative	Negative
Fiche's test	Negative	Negative
Specific gravity	1.352g/ml	1.352-1.358g/ml
Moisture content	20.4%	≤ 23%
Refractive Index	1.485	1.474-1.504
Apparent reducing Sugar	68.9%	≥ 53%
Apparent Sucrose Content	13%	≤ 15%
Water Insoluble Solid Content	0.20	≤ 0.5
Mineral Content (Ash)	0.11%	≤ 1.0%
Acidity	20 me acid per 1000g	≤ 40 me acid per 1000g
Diastase Activity	5.5	≥ 3
Hydroxylmethyl Furfural Content	78 mg. per kg	≤ 80 mg. per kg

Control Drug

The control group was treated with Triple Antibiotic Ointment with the Pain Relievers. Composition of 1g of ointment is given below.
Bacitracin USP – 500 Units
Neomycin Sulfate USP – equivalent to Neomycin 3.5mg
Polmyxin B Sulfate USP – 5000 Units
Lidocaine USP – 40mg

The antibacterial action of bacitracin is mainly against gram positive bacteria and few of the gram negative bacteria, while that of neomycin is against gram negative bacteria and partially against gram positive bacteria. The antibacterial spectrum of polymycin B is almost exclusively limited to gram

negative bacteria. The combination of these antibiotics is used for the treatment of minor skin infections caused by small cuts, scrapes or burn. These characteristics make the test drug ideal for the comparison with the control drug and fulfills the objective behind the research study.

Data Analysis

SPSS 22 software was used for data analyses. The day at which the Wound Score become 13 was regarded as the day of Complete Healing according to the Bates-Jensen Wound Assessment Tool. Mean and Standard deviation were calculated for all quantitative variables. Frequency and percentages were calculated for all qualitative variables. Results were displayed using frequency tables and bar charts. The second level of analyses was cross-tabulations. Chi square test was used to investigate the statistical significance of the association between two qualitative variables. The level of the significance (α) was set to be 0.05. Results with p-value less than 0.05 were considered statistically significant.

Ethical Approval

Ethical Approval was obtained from Hamdard University Ethics Committee in written. Informed written consent was obtained from patients and the patients are given the right to withdraw at any time during the course of the trial. Identity will not be revealed and the data would be kept strictly confidential.

Results

Total 196 subjects were enrolled in the study on the basis of selection criteria and were distributed equally in both groups by randomization i.e. Test Group contains n=98 (50%), and similarly, control Group also contains n=98 (50%). There was no patient who failed to continue the follow up.

For each subject, the variables for wound assessment were summed up to calculate the stage of Wound Healing. The total rating score for each visit of every individual participating in the study was plotted on the Wound Status Continuum of the Bates-Jensen Wound Assessment Tool. The Mean value of the Baseline Wound Status Continuum for test group was 24.85, and for control group it was 25.13. The mode value coincide with the median value and was same for both groups i.e. score 25 (30.61%), so the range for both groups was up to 18 with the same minimum and maximum values for both test group and control group. Chi-Square test was applied and the P-Value was calculated to be 0.414 showed insignificant baseline difference between the Wound Status Scores of the two groups.

There was a gradual reduction in size of the wound with each 5 days intervals, in both groups from the baseline size (P-value = 0.872). There was an appreciable difference in the reduction of size among the test group at day 5 with a P-value of 0.028 which is statistically significant. At day 10, there was a very striking improvement within the test groups in terms of reduction in the size of wound, with a P-value of 0.007 which is highly significant.

Culture samples from wounds were taken at three occasions, one at the time of allocation, other at day 15 and the last at day 30. Baseline Culture Samples yield, *Pseudomonas aeruginosa* in 58 (29.59%) subjects with highest frequency, MSSA in 51 (26.02%) subjects, *Staphylococcus* (spp. not specified) in 38 (19.38%) subjects, MRSA in 27 (13.77%) subjects and *streptococcus* in 22 (11.22%) subjects. (Fig. 1) Chi-square test was applied to evaluate the difference of baseline organisms among the test and the control group and P-Value was found to be 0.231 which is not significant.

When comparing the two groups at day 15, *Pseudomonas Aeruginosa* persist in just 2 samples of test group with a total reduction of 93.34% while 82.15% reduction in control group. The mean healing time was 14.37 days for the test group while it is 17.64 days for the control group. (Fig. 2).

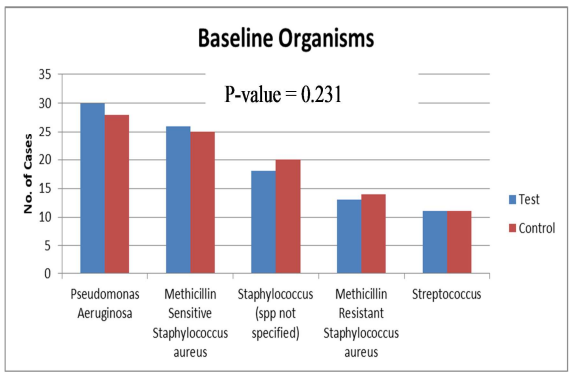

Fig 1. Baseline organisms.

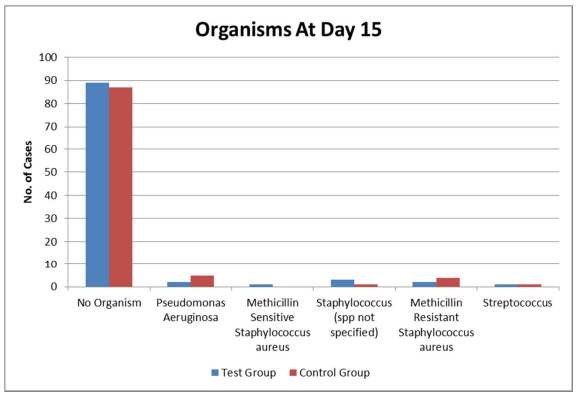

Fig 2. Organisms in the two groups at day 15.

At day 15, there was 100% reduction of MSSA in control group while 96.16% in test group but the mean healing time was 11 days for the test group, while it was 11.12 days for the control group. (Fig. 2)

Also there was 95% reduction of *Staphylococcus aureus* of which species not identified, in control group while 83.34% in test group at day 15. The mean healing time for *Staphylococcus aureus* of which species not identified, was 14.61 days for the test group, while it was 15.10 days for the control group. (Fig. 2)

At day 15, there was 84.62% of MRSA in test group while only 71.43% reduction in control group. The mean healing time was 18 days for the test group while it was 20.29 days for the control group. There was 90.91% reduction of *Streptococcus* in both the groups at day 15. (Fig. 2)

The mean healing time for the test group was calculated to be 14.06 day, while that for the control group was 15.65 day.

Discussion

The database providing research on the subject of efficacy of honey as topical anti-bacterial agent for the treatment of skin wounds was very vast but still there is lacking of controlled trials. Furthermore, there was no single controlled trial ever conducted which solely involved infected wounds that were acute in nature. So, data providing research based on controlled trials on the topic of honey for the treatment of minor acute skin lesions with respect to infected wounds, was very scarce and insufficient to gather enough information that was relevant to the subject under investigation. Hence, there was immense difficulty in comparing the results with the previous studies held to determine the efficacy of honey for the treatment of infected acute wounds with the control group. Nevertheless, there were a huge number of controlled trials that compared honey for the treatment of burns and also for the chronic wounds such as venous ulcers and diabetic foot.

In this study, the allocation of patients in the test and control groups, were based on randomization by 'chit method', so the baseline difference, when comparing different parameters, between the test and the control group, was negligible. The difference in the organisms isolated at the baseline was also negligible among the two groups. The variables used for wound assessment make the two groups ideal for the comparison and removes the biasness of results.

The wounds in the test group that were treated with honey, achieve much greater reduction in size as compared to the control. There were not enough studies on the database, which compared the size of the wounds between the honey and the control drugs for the acute infective wounds.

The results of this study regarding the prevalence of organism are consistent with the other studies. *Staphylococcus aureus*, including MRSA and MSSA, was the most common organism (n=78), followed by *Pseudomonas aeruginosa* (n=58).

There is a difference of 3.27 days for the mean healing time so honey seems to be more effective on *Pseudomonas aeruginosa* but the results are not significant statistically (P-value = 0.068). The results goes in favor of honey for MRSA as there is a difference of 2.29 days for the mean healing time but again the results are not significant when chi-square test was applied (P-value = 0.502).

Other controlled trials of honey also report greater proportions of negative swabs in the honey group as compared to the control group at day 7, but the evidence of infection in minor acute wounds was very scarce and could not be compared with this study.

There was 1.59 day difference in favor of honey for the treatment of acute infective wounds. In spite of obvious rapid healing time for honey, the difference was not enough to be statistically significant when the chi-square test was applied (P-value is 0.305). Fortunately, there is satisfying data from other controlled trials referring to the difference in the mean healing time between the honey group and the controls demonstrating rapid healing time for the honey group.

Honey not only reduces the healing time required for the complete healing of infective wounds but also increases the cost effectiveness. Moreover, it does not aid in the development of resistant organisms which greatly hinders the healing of wounds. It is easily available for the patients living in remote areas and also very feasible for application. Honey could be an excellent substitute for other conventional dressing required for the wound healing and a good alternative to anti-bacterial agents required for the infection control. Therefore, the implementation of its use for the treatment of acutely infected wounds must be reinforced by the physicians, especially in the less privileged areas of the developing countries. The clinical trial based research for the use of honey in the treatment of acutely infected wounds is lacking, so there is need of more evidence based controlled trials for the implication of its better use.

References

Al-Jabri AA. 2005. Honey, milk and antibiotics. African Journal of Biotechnology **4(13),** p. 1580-7.

Baghel PS, Mathur RK, Randa R. 2009. A comparative study to evaluate the effect of honey dressing and silver sulfadiazene dressing on wound healing in burn patients. Indian Journal of Plastic Surgery **42(2),** p. 176-81.

Bessa LJ, Fazii P, Di Giulio M, Cellini L. 2015. Bacterial isolates from infected wounds and their antibiotic susceptibility pattern: some remarks about wound infection. International Wound Journal **12(1),** p. 47-52.

Biswas ABM, Hurst C, Gruessner R, Armstrong D, Rilo H. 2010. Use of sugar on the healing of diabetic ulcers: a review. Journal of Diabetes Science and Technology **4(5),** p. 1139-45.

Cutting KF. 2018. The current burden of infected wounds. British Journal of Healthcare Management **22(9),** p. 436-8.

Esmon CT. 2004. Crosstalk between inflammation and thrombosis. Maturitas **47(4),** p. 305-14.

Garten AJ. 2017 Assessing And Monitoring Wound Progression With The Bates-Jensen Wound Assessment Tool.

Gethin G. 2009. Manuka honey vs. hydrogel--a prospective, open label, multicentre, randomised controlled trial to compare desloughing efficacy and healing outcomes in venous ulcers. Journal of Clinical Nursing **18(3),** p. 466-74.

Giacometti ACO, Schimizzi AM, Del Prete MS, Barchiesi F, D'Errico MM, Petrelli E, Scalise G. 2000. Epidemiology and Microbiology of Surgical Wound Infections. Journal of Clinical Microbiology **38(2).**

Huddleston H. 2014. Obtaining a wound swab culture specimen. Nursing **44(7).**

Ingle JL, Polindern K. 2006. Wound healing with honey – a randomised controlled trial. South African Medical Jounal **96(9),** p. 831-5.

Jull AB, Cullum N, Dumville JC, Westby MJ, Deshpande S, Walker N. 2015. Honey as a topical treatment for wounds. Cochrane Database of Systematic Reviews 3.

Majtan JKP, Majtan T, Walls AF, Klaudiny J. 2010. Effect of honey and its major royal jelly protein 1 on cytokine and MMP-9 mRNA transcripts in human keratinocytes. Experimental Dermatology **19(8),** p. 73-9.

Malik KI, Aslam A. 2010. Honey compared with silver sulphadiazine in the treatment of superficial partial-thickness burns. International Wound Journal **7(5),** p. 413-7.

Mashhood AA, Sami AN. 2006. Honey compared with 1% silver sulfadiazine cream in the treatment of superficial and partial thickness burns. Journal of Pakistan Association of Dermatologists **16,** p. 14-9.

Memon AR, Imdad A, Memon GA. 2005. Therapeutic effects of honey versus silver sulphadiazine in the management of burn injuries. Journal of Liaquat University of Medical and Health Sciences **4(3),** p 100-4.

Molan PC, Rhodes T. 2015. Honey: A Biologic Wound Dressing. Wounds **27(6),** p. 141-51.

Molan PC. 2002. Re-introducing honey in the management of wounds and ulcers - theory and practice. Ostomy Wound Management **48(11),** p. 28-40.

Molan PC. 2009. Debridement of wounds with honey. Journal of Wound Technology **5,** p. 12-7.

Struelens MJ. 2006. Rapid identification of methicillin-resistant Staphylococcus aureus (MRSA) and patient management. Clinical Microbiology and Infection **12,** p. 23-6.

Subrahmanyam M. 1991. Topical application of honey in treatment of burns. The British journal of Surgery **78(4),** p. 497-8.

Subrahmanyam M. 1993. Honey impregnated gauze versus polyurethane film (OpSiteR) in the treatment of burns - a prospective randomised study. British Journal of Plastic Surgery **46,** p. 322-3.

Subrahmanyam M. 1994. Honey-impregnated gauze versus amniotic membrane in the treatment of burns Journal of the International Society for Burn Injuries **20(4),** p. 331-3.

Subrahmanyam M. 1996. Honey dressing versus boiled potato peel in the treatment of burns: a prospective randomized study. Journal of the International Society for Burn Injuries **22(6),** p. 491-3.

Subrahmanyam M. 1998. A prospective randomised clinical and histological study of superficial burn wound healing with honey and silver sulfadiazine. Journal of the International Society for Burn Injuries **24(2).**

Tasleem S, Khan SA, Hashimi K. 2011. Honey ointment': A natural remedy of skin wound infections. Journal of Ayub Medical College **23(2).**

Tasleem S, Khan SA, Hashmi K. 2013. Efficacy of newly formulated ointment containing 20% active antimicrobial honey in treatment of burn wound infections. Journal of Ayub Medical College **25,** p. 145-8.

WHO. 2013. Traditional Medicine Strategy 2014-2023. Available from: http://www.who.int /medicines/publications/traditional/trmstrategy1423 /en/. Cited on 23rd May 2019