ORIGINAL ARTICLE

EFFICACY OF NEWLY FORMULATED OINTMENT CONTAINING 20% ACTIVE ANTIMICROBIAL HONEY IN TREATMENT OF BURN WOUND INFECTIONS

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Background: Honey has been familiar to possess antimicrobial potential to clear infection against burn wound infecting bacteria since ancient times. The objective of the study was to evaluate the efficacy of the newly formulated honey ointment during the treatment of burn wound infections. The Experimental (Non comparative) study was conducted at outpatient department of Dermatology, Fauji Foundation Hospital, Rawalpindi from November 2009 to October 2010. **Methods:** The antimicrobial activity of different Pakistani floral sources (*Acacia nilotica species indica*, *Zizyphus, Helianthus annuus* and *Carissa opaca*) honey samples were investigated by disc diffusion method against freshly isolated burn wounds infecting bacteria. Ointment containing 20% active antimicrobial honey was formulated as a sovereign remedy. A total number of twenty patients with second degree of burn wounds on different parts of the body were studied. A thin layer of honey ointment on gauze was applied to the wounds two to three times a day up to the complete healing. **Results:** During microbiological study, Pakistani honey samples were discovered to exhibit a very promising antimicrobial activity against all the wound infecting microorganisms tested. Clinical trials demonstrated that the topical application of honey ointment have significant control of infections arising form pathogenic bacteria and up to 100% healing results were observed in all burn wound cases within mean healing time for the duration of 8.15 (3–18) days time period. **Conclusion:** Newly formulated ointment containing 20% active antimicrobial honey is more effective and low-cost alternative preparation for the treatment of burn wound infections.

Keywords: Antimicrobial activity of honey, Honey ointment, Burn wound infections.

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INTRODUCTION

The frequency of bacterial resistance to all kind of antibiotics is increasing across the globe, thus the researcher utmost aim is to investigate the efficacy of alternative preparation for the welfare and benefit of humanity. Honey has been familiar to possess antimicrobial potential to clear infection against burn wound infecting bacteria. United State FDA authority also gave clearance of Medi honey as wound dressing product.[1]

Subrahmanyam[2] collected cultural swabs from 80 burn patients and isolated Coagulase negative *Staphylococcus*, *Pseudomonas aeruginosa*, Coagulase positive *Staphylococcus*, *Citrobacter diversus*, *Citrobacter freundii*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Escherichia coli* and *Proteus vulgaris* from burn wounds and observed 100% inhibition of these isolates on Mueller Hinton agar with honey. The promising antimicrobial results of honey against the pathogenic bacteria led to the fact that honey could be successfully applied for the treatment of infection of wounds arises from pathogenic bacteria.[3]

There is no significant evidence in favor of the silver containing products in preventing or promoting wound healing.[4] Yusof *et al*[5], applied dressing on burn wounds of rats (Sprague-Dawley) and found that the application of radiation sterilized Gelam (Malaysia) honey significantly enhanced the rate of healing and reduced the inflammation of burning than silver sulfadiazine cream. Antimicrobial activity[6,7], high osmolarity[8], low *p*H[9,10]and hydrogen peroxide[11] of honey altogether contribute a combined effect to clear infection in burn wounds and thus enhance the wound healing.

In brief, having vision that honey has antibacterial[2,6,7] and anti-inflammatory properties[5], it lessen the risk of cross infection[12] and promotes wound healing[5,13] ,the present study was assigned to optimize this natural product for the benefit of humanity. The objective of the present study was to evaluate the efficacy of the honey ointment during the treatment of burn wound infections.

MATERIAL AND METHODS

Four honey samples of different floral sources of Pakistan namely (*Acacia nilotica species indica*, *Zizyphus, Helianthus annuus* and *Carissa opaca*) were investigated by CLSI (formerly NCCLS) reference disc diffusion (Kirby Bauer) method against freshly isolated microorganism (*Staphylococci aureus, Staphylococci epidermidis, Streptococcus faecalis*, *Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli, Proteus vulgaris* and *Candida albicans*) from burn wound patients and *Staphylococci*

aureus ATCC 6538, *Pseudomonas aeruginosa* ATCC 9022 , *Escherichia coli* ATCC 25922 , *Candida albican* ATCC 15146.

Crude honey (antimicrobial potential) 200gm and other ingredients: White soft paraffin (Merck) 100gm, Liquid paraffin (Merck) 100gm, Lanolin (Merck) 100 gm were used for the formulation of ointment, and take it up to the level 1000gm with white soft paraffin. An effective dose of gamma radiation was applied to sterilize the product. The ointment was store at room temperature (5–25 °C).

To evaluate the efficacy of ointment containing 20% active antimicrobial honey on burn wound cases. Clinical trials were conducted under the supervision of doctors at Fauji Foundation hospital Rawalpindi from November 2009 to October 2010. The protocol of study was approved by the Research Committee, Department of Pharmaceutics Faculty of Pharmacy, University of Karachi. It was non comparative experimental study. All patients were thoroughly informed about the efficacy and safety of honey ointment under trial. Patients submitted their consent in writing before inclusion. Patients who could not respond on due date or applying any other treatment along with topical honey treatment were excluded.

Clinical history, visual observation, available data, laboratory testing of informative material was used for the diagnosis. First wound was treated with normal saline then dressing with honey ointment on gauze was applied on the wound two to three times a day up to the complete healing as part of clinical trial. Clinical assessment was made at alternate day during treatment under the supervision of doctor on research proforma. An evaluation response in to the treatment was assessed in accordance with the criteria of visual observation. Wound is considered "closed and healed" if the repairs of the epidermis due to the outgrowth of epithelial cells at the edge of wounds are observed. Size of wound was measured in mm^2 by tracing infection boundaries on a transparent paper before and after treatment. For medical attention Total body surface area (TBSA) as an assessment measure of burn wound also determined in Table-3. The healed area is calculated by subtraction the wound area after treatment from the area of wound before treatment as calculated by Rozaini et al.[14] Wound healing percentage was calculated by using the following formula of Baie and Sheikh.[15]

$$\% \text{ of wound contraction} = \frac{\text{Healed Area}}{\text{Total area of the wound before treatment}} \times 100$$

RESULTS

Demographic features of (n=20) burn wound patients under trial have been illustrated in Table-1. The Table-2 illustrates the results of investigation by CLSI (formerly NCCLS) reference disc diffusion (Kirby Bauer) method against ATCC microorganism and freshly isolated clinical isolates from burn wound patients. In our clinical trial we experienced a total number of 20 (10 male and 10 female) second degree of burn wounds cases. These patients had visited the clinic with burn wound infection on different part of the body, had age range of 1–49 years with Mean±SD of 23.85±17.088 years. Most of these patients were employed and doing their job. Most of the females were house hold. The application of newly formulated honey ointment showed very promising results and up to 100% healing results were observed in all burn wound cases within mean healing time for the duration of 8.15 (3–18) days time period Table-3. Pictures show representative cases before and after treatment.

Table-1: Demographic features of patients (=20)

Parameter		Number
Sex	Male	10
	Female	10
Locality	Developed	15
	Undeveloped	5
Family Size (number of members)	2	3
	3	10
	6 and above	7
Education	Literate	15
	Illiterate	5
Occupation	Job	5
	Business man	1
	House hold	7
	Others	7
Monthly income	Low	2
	Medium	16
	High	2

Table-2: Antimicrobial activity of honey samples of different floral sources of Pakistan against standard micro-organisms and clinical isolates

Microorganism	*Acacia nilotica species indica* Honey	*Helianthus annuus* Honey	*Carissa opaca* Honey	*Zizyphus* Honey
	Diameter of Zone of Inhibition (mm)			
Staphylococcus aureus	27.333±0.333	20.0±0	35.0±0	30.0±0
Staphylococcus epidermidis	23.333±0.333	17.333±0.333	35.333±0.333	19.667±0
Streptococcus faecalis	17.0±0	17.333±0.333	19.333±0.333	17.6667±0.333
Pseudomonas aeruginosa	21.667±0.333	18.333±0.333	18±0	23.0±0
Klebsiella pneumonia	19.0±0	14±0	17±0	19.333±0
Escherichia coli	25.667±0	18±0	29.333±0	29.0±0
Proteus vulgaris	17.333±0	17.333±0.333	17.333±0	27.0±0
Candida albican	18.0±0	12.0±0	16.333± 0	17.333±0.333
Staphylococcus aureus ATCC 6538	16.7±0.3	19±0	32±0	18.3±0.3
Pseudomonas aeroginosa ATCC 9022	16.3±0.3	17±0	17±0	17±0.3
Escherichia coli ATCC 25922	19±0.6	17±0	17±0	16.3±0.3
Candida albican ATCC 15146	13±0	10±0	12±0	13±0.6

Value represent mean of triplicate determination ±SE

Table-3: Clinical evaluation of the efficacy of newly formulated honey ointment on burn wounds treatment
(n=20, II degree Burn wound)

Case	Cause of burning	Site	TBSA (m^2)	Wound area before treatment mm^2	Healed area mm^2	Healing %	Healing duration (days)	Mean Healing duration (days)	Range (days)
B.1	Hot water	Right hand & legs	0.73	13600	13600	100	10	8.15±4.29	3–18
B.2	Motor bike silencer	Right Leg	1.79	1850	1850	100	3		
B.3	Hot curry	Left hand & fingers	0.82	9856	9856	100	3		
B.4	Steam	Right Leg	1.62	18750	18750	100	10		
B.5	Car radiator stream	Right Hand	1.71	14000	14000	100	7		
B.6	The motor bike silencer	Right Heal	1.86	264	264	100	5		
B.7	Gas flam	Left Thigh	0.83	33401	33401	100	8		
B.8	Hot water and burner	Right arm & hand	1.60	18240	18240	100	5, 16*		
B.9	Electric current	Both sole	1.82	8401	8401	100	15, 18*		
B.10	The motor bike silencer	Wrist	1.76	286	286	100	5		
B.11	Steam	Wrist	1.66	13230	13230	100	14		
B.12	Motor bike silencer	Right Leg	1.49	15750	15750	100	10		
B.13	Hot Iron	Left Wrist	0.44	748	748	100	4		
B.14	Hot metal of gas burner	Right Wrist	0.43	2018	2018	100	6		
B.15	Hot water	Left Leg	1.36	17696	17696	100	7		
B.16	Hot water	Right Foot	1.43	5494	5494	100	6		
B.17	Cocking oil	Right middle finger	1.61	320	320	100	4		
B.18	Hot water	Chest	0.55	9360	9360	100	12		
B.19	Hot water	Left Foot	1.49	5130	5130	100	10		
B.20	Hot water	Hand and Foot	0.40	2242	2242	100	5		

Note: B=Burn, TBSA=Total body surface area, *Healing time of different wounds of patient number B.8 and B.9.
*DuBois D, DuBois DF. A formula to estimate the approximate surface area if height and weight be known. Arch Int Med 1916;17:863-71


Case B1: Before Treatment


B1: After Treatment with Honey Ointment


Case B5: Before Treatment


B5: After Treatment with Honey Ointment


Case B7: Before Treatment


B7: After Treatment with Honey Ointment


Case B11: Before Treatment


B11: After Treatment with Honey Ointment

DISCUSSION

Pakistani honey samples were discovered to exhibit a very promising antimicrobial activity against ATCC microorganism and freshly isolated burn wounds infecting bacteria. These results are in confirmation with the previous studies.[16,2,17] The incorporation of such standard honey in formulation authenticated the effectiveness of wound care product. Similar finding was earlier reported that honey is effective against *Pseudomonas aeruginosa.*[18]

Application of ointment containing 20% active antimicrobial honey to infected site of burn wounds was very effective. The reported signs, symptoms and skin condition of the patient, e.g., colour, appearance (blister), swelling/oedema, pain, inflammation, infections were gently managed in the early 3–5 day application of newly formulated honey ointment dressing. Burn wound is an oxidative injury with intensified activity of oxygen free radical and the painfulness of wounds resulted from factors released in the inflammatory response sanitising nerve endings, the occurrence of these factors was monitored through the anti-inflammatory and antioxidant property of the newly formulated honey ointment. Subrahmanyam[19] also reported the pain relieving and soothing action of honey in treatment of burns and scalds. Present clinical trial

demonstrated that the topical use of honey ointment had significant progression control of skin zone in necrosis after burn, disinfected the wound and showed a more rapid reduction in size of wound.

Clinical evaluation of patients was undertaken on alternate days. Even at last follow-up no infection was noted in all patients and no patient required systematic antibiotics. The rate of wound healing was associated with presence of foreign bodies and microorganisms, wound size, blood supply to the wound site, age, health and nutritional status of patients.[20] The present results are in confirmation with Subrahmanyam[2,21] reported that honey was more active in management of infection and enhanced the rate of healing of burn wounds compared to silver sulfadiazine. Bardshaw and Claire[22] also observed no significant difference between honey, iodine and silver against wounds infected with *Escherichia coli, Staphylococcous aureus* and *Pseudomonas aeruginosa*. Thus honey dressing improves wound healing and sterilises the wound compared to silver sulfadiazine dressing.[23]

The ointment containing 20% active antimicrobial honey controls the local stinging at wound site and ensures the active ingredient to persist at wound site for longer duration. Like modern dressing it offers a gentle approach in application: easy to apply, no adherence to wound, removed painlessly without any adverse effect to the site of application, and creates a moist wound healing environment which appeared to be favourable to accelerate wound healing. In the present clinical trials doctors and dermatologist judged the severe effectiveness very keenly and assigned a valued status to honey in the treatment of infected burn wounds. Al-Waili *et al*[24] encourage the use of honey in clinical practice. Song and Salcido[25] also reported the significant effect of application of honey in the treatment of burn wounds. However further research work for randomized control trial should be done to access a brilliant success towards original alternative antimicrobial treatment of burn wound.

CONCULSION

Newly formulated ointment containing 20% active antimicrobial honey is alternative low-cost preparation. It was outstanding rather than effective and soothing for the treatment of burn wound infections. It provides a broader range of antimicrobial and anti-inflammatory properties. It develops a moist wound environment and stimulates healing processes.

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