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ORIGINAL ARTICLE

Evaluation of wound healing efficacy of Honey (Berseem variety) in treating Diabetic foot ulcers

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ABSTRACT
Successful diagnosis and management approach needs holistic approach specifically effective local wound care. Honey is a natural product with well-established anti-microbial function and its ability to promote wound healing with safety profile. To investigate the wound healing efficacy of honey in diabetic foot ulcers. In this prospective, randomized, controlled study, 80 subjects with type 2 Diabetes mellitus subjects were randomly allocated either to honey dressing(experimental)or saline dressing(control). Post wound debridement DFU were assessed before and after treatment intervention dressings. Base line demographic characteristics along with biochemical parameters were matched in the two groups. Post topical dressing intervention showed gradual reduction in mean RBS as well as mean wound area. On 60th day, percentage wound contraction significantly high in honey group along with shorter healing time (74.5±14 days) in honey group compared to (89.3±21.0 days) saline group. The study proved that compared to saline, the honey (Berseem variety) dressings demonstrated better efficacy with respect to percentage wound contraction as well as duration of wound healing. Thus, honey dressings can be utilized as safe, cost effective, easily available adjuvant to treat DFU.
***Key words**: Honey, saline dressing, Diabetic foot ulcer, Wound healing.*

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INTRODUCTION

Diabetes mellitus is a worldwide health problem with linked complications of peripheral neuropathy, peripheral vascular disease, and immunopathy. Chronic diabetes mellitus makes the feet more susceptible to injury and the development of non-healing wounds. Most wounds are delayed healing, have significant bioburden, and also may harbour resistant bacterial strains [1,2]. According to epidemiological data, 4.5 per 1000 Indians and 6 million globally suffer from chronic wounds. One-third of infected diabetic foot ulcers result in limb loss, with 2-year survival rate of 50-60% [3]. Rigorous wound management and suitable dressings are crucial in preventing limb loss, as existing treatments may be expensive or useless in indolent ulcers with multidrug-resistant biofilms [4]. Poor incorporation of tissue-engineered novel matrix therapies in the previous two decades led to mainstreaming old and traditional medicines.

Honey is one of the best dressing materials used since ancient times to heal wounds, which has been rediscovered with clinical and research-based evidence to establish its usefulness again with emergence of antibiotic resistant strains [5-7].

Honey is widely available in nature, and it has been approved by WHO for topical use in wound healing It has been found that all honeys are not equal and different bio-active components are present in honeys from various floral sources [8]. Manuka honey obtained from specific floral sources in forests of New Zealand, Australia (Leptospermum species) has enhanced anti-bacterial activity, most well researched

and widely marketed as medicated honey [9]**.** No study has till now been conducted on Indian 'Berseem honey' for it's wound healing potential and the objective of the present study is to explore it's utility as wound healing agent in patient with diabetic food ulcer.

MATERIAL AND METHODS

This prospective study was conducted at Department of General surgery, Gajra raja Medical College, JA group of Hospital Gwalior (M.P.) & Gwalior Diabetes & Foot Care Center from June between 2018 to Oct 2020.

Procurement, Sterilization and preparation of honey dressings –

Honey (Raw Berseem organic honey) was procured from Integrated Bee Keeping Center and Zonal Agriculture research station (Raj Mata Vijaya Raje Scindia Krishi Vishwavidyalaya Center Morena, Gwalior (M.P.)

Recruitment of subjects:

A total of 80 type 2 diabetes mellitus subjects of age between 30- 80 years and either gender presenting with foot ulcer were recruited for the study following inclusion-exclusion criteria laid down in Table-1.

Table 1: Inclusion and exclusion criteria of the study subjects

Inclusion criteria	EXCLUSION CRITERIA
• Type 2 diabetic patients with foot ulcer. • Wound categorization by University Texas classification- only Grade (1-2) stage (B) ulcers. • Patients willing to participate in the study. • Surgical emergency (Foot attack) • Diabetic neuropathy and retinopathy	• Critically ill Pts (unfit for anaesthesia, surgery) • ABI< 0.9, Peripheral obstructive vascular disease (Burger's disease, Raynaud's disease, DVT) • Existing mental illness. • Non-diabetic neuropathic foot ulcers • Bleeding diathesis, deranged coagulation profile. • Diabetic nephropathy, severe anaemia, hypoproteinaemia.

Pre-experimental parameters documented:

After admission, all the data was recorded on predesigned proforma, and an informed consent was taken before commencement of study. Anthropometric measurements were taken; overall clinical assessment of patients along with foot ulcer assessment were carried out. Assessment of Neuropathy (Biothesiometer, Monofilament test), Limited joint mobility, Ankle brachial index (ABI) and Plantar pressure measurement (Harris ink mat) were also done.

Experimental Design:

Randomized, case control, single blinded, experimental study design was followed.

According to the experimental design, subjects were randomly divided into two equal groups and were allocated to one of the following two groups:

- Group A-Standard Normal Saline dressings (control group). N=40
- Group B-Honey dressings (experimental group), N=40

The study protocol involved the following steps:

1. Washing the foot thoroughly before each treatment with antiseptic solution and measurement of wound dimensions (length, width, depth) with the help of sterile tape gauze, and wound area traced on graph paper.
2. Assessment of condition of foot, pedal pulsations, skin colour, local warmth and swelling was done. Wounds were then classified based on University of Texas classification system
3. Collection of blood samples on days 0 and 30th day for biochemical analyses
4. Full thickness wound biopsy including small portion of normal skin was taken from edge of the wound (control)on day 0 and 30th day for histopathological examination (H&E stain).
5. Sharp debridement of necrotic tissue (in operation theatre /ward) to promote healthy tissue granulation. The deep tissue is subjected to culture sensitivity tests.
6. Application of the appropriate dressings (interventions)Honey /Saline impregnated gauze to cover wound bed and secondary dressings applied.
7. Empirically patients were started on broad spectrum antibiotics, later culture specific antibiotics were administered. All patients were kept on Insulin and oral hypoglycaemic agents for glycaemic control.

8. Wound dimensions were measured at 10day intervals during treatment.
9. Offloading (walker, rocker bottom shoes, diabetic sandals) and home dressings instructions were addressed to the patient. Complete healing was defined as epithelialization of the wound.

Biochemical analyses

Whole blood samples were assessed for total leucocyte count, blood glucose, HbA1c. Plasma samples were analyzed for Lipid profile, Renal function and Liver function tests.

Estimation of antioxidants in wound tissue:

Tissue biopsies were used for determination of tissue oxidative stress parameters (Glutathione reductase, catalase, Superoxide dismutase). Superoxide dismutase was assayed as per the method described by Winterbourn (1993). Catalase estimation was done as per Aebi (1984) and Glutathione estimation was based on reduction of 5,5- dithio bis (2- nitro benzoic acid) according to Ellman, (1959) [10-12]. This study protocol was approved by Institutional ethics committee of Gajra raja Medical College, Gwalior. (No.-22866-68).

RESULTS

The study subjects included both males (N=55) and females (N =25). The mean age of the study population was observed 55.7 years and there was no significant different between study groups.

Table 2: Frequency and mean distribution of age and gender of the study subjects in various treatment groups

Treatment	N (M,F)	Mean age	BMI kg/m^2	Blood Pressure mmHg	
				SYS *	DYS
Honey	40 (28,12)	54.25 ±8.2	27.93 ±3.9	142±8.1	81.4±9.3
Saline	40 (27,13)	57.08 ±9.4	28.12 ±5.3	140.8 ±5.1	80.9 ±9.5
Total	80 (55,25)	55.7 ±8.8	28.02 ±4.6	142.4 ±9.8	82.4 ±8.9

Values are expressed in Mean ± SD, * p value p=<0.05.

Basic and anthropometric parameters of the study population were ascertained and the mean age being 54.25 years in honey group and 57.08 years in saline study group. Maximum patients (29) were in the age group 41-60 years. No significant differences were found between study groups. Based on the BMI the mean was found to be27.93 kg/m^2 in honey group and 28.12kg/m^2 in saline group. Mean Systolic blood pressure (SBP) was 142 mmHg and 140.8 mmHg in honey and saline group respectively. These values are found significantly different (p= <0.05). Diastolic blood pressure (DBP) was found to be 81.4 and 80.9 mmHg in honey and normal saline group respectively, No significant differences was found between study groups based on Diastolic BP (p =0.89).

Based on the diabetes duration the mean duration of diabetes was found 11.6 and 13.9 years in honey and saline group respectively and found no significant difference between both groups (p =0.76). (Table: 3).

Table:3 Frequency and Mean distribution of study subjects in various treatment groups based on diabetes duration

Treatment	N	Mean (Years)	Std. Deviation
Honey	40	11.6	5.7
Saline	40	13.9	7.6
Total	80	12.75	6.65

The systematic finding of the signs and symptoms in the subjects were plotted in Figure 1. Overall, symptoms like pain (75%), burning (28%), Itching (26%) were reported by the study subjects. During the physical examination in the wound area signs like odour (46 %), discharge (46 %), redness (45 %) and swelling (51 %) were observed.

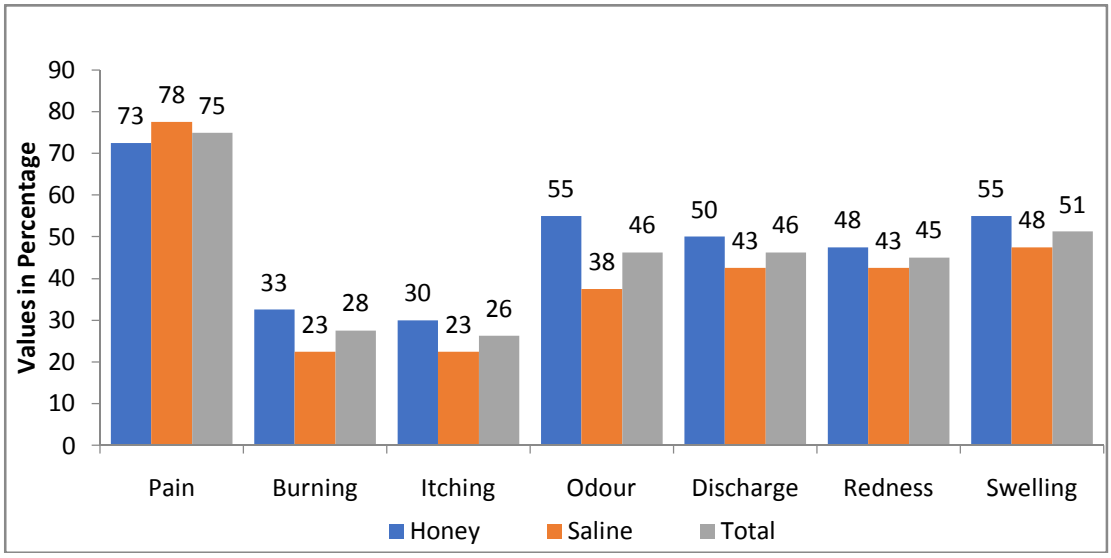


Figure 1: Signs and Symptoms of the study subjects in different treatment groups at baseline visit.

Table 4: Glycemic parameters of the subjects in different treatment groups

Treatment	FBS mg/dl	HbA1c* %	C-peptide ng/ml	T. Cholesterol mg/dl	Triglycerides mg/dl	HDL-C* mg/dl
Honey	279.6± 68.7	7.54±1.13	1.77±0.73	209.7±32.8	179.2± 36.9	53.1± 8.1
Saline	275.5 ± 68.8	7.8±0.75	1.65±0.78	206.8± 39.4	196.6± 44.6	45.5± 5.1
Total	277.6± 68.8	7.7±0.9	1.7±0.8	208.3± 36.1	187.9± 40.8	49.3± 6.6

Note: Values are expressed in Mean ± SD, * p value p=<0.05.

Based on the glycemic and lipid parameters, FBS, HbA1c, c-peptide levels, total cholesterol level of subjects in treatment groups were found to be higher in honey group compared to saline group. However statistically not significant except HbA1c and HDL-C (p = < 0.05)

Liver function and kidney function markers were monitored in all the study subjects following enrolment and found no significant difference between both groups except the SGOT values (p=<0.05) (table: 5)

Table 5: Liver and kidney function profile among the study participants

Parameters		Honey	Saline	Total
SG0T * (IU/l)	Mean	59.5	56.7	58.1
	SD	14.4	11.4	12.9
SGPT (IU/I	Mean	60.9	57.5	59.2
	SD	16.4	12.6	14.5
S. Bilirubin (mg/dl)	Mean	1.6	1.7	1.7
	SD	0.74	0.72	0.7
PT (min)	Mean	12.5	12.4	12.5
	SD	0.96	0.88	0.9
Urea (mg/dl)	Mean	40.8	39.5	40.2
	SD	8.8	9.6	9.2
Uric acid (mg/dl)	Mean	5.3	5.6	5.5
	SD	1.3	1.2	1.3
Creatinine (mg/dl)	Mean	1.2	1.3	1.3
	SD	0.7	0.9	0.8
S. Na+ (mEq/L	Mean	128.6	136.9	132.8
	SD	5.6	6.2	5.9
S. K+ (mmol/L	Mean	3.9	4	4.0
	SD	0.6	0.5	0.6

Note: Values are expressed in Mean ± SD, * p value p=<0.05.

Table 6: Tissue antioxidants levels of study subjects

Treatment	SOD (U/min/mg protein)	GSH (U/min/mg protein)	Catalase (U/min/mg protein)
Honey	35.8 ± 7.4	0.76 ± 0.26	6 ± 2.6
Saline	39.3 ±8.9	0.8 ± 0.24	5.7 ± 2.7
Total	37.55 ± 8.15	0.78 ± 0.25	5.85 ± 2.65

Note: Values are expressed in Mean ± SD,

Tissue antioxidants enzyme like SOD GSH and Catalase on wound tissue were analyzed on the beginning of the study and found no significant different between the study groups

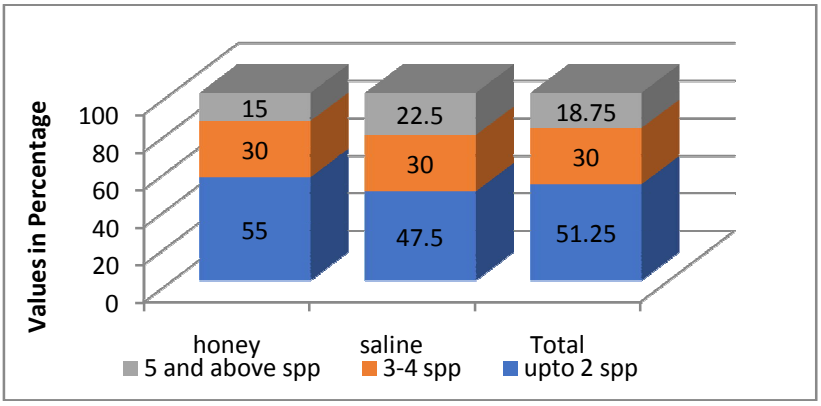
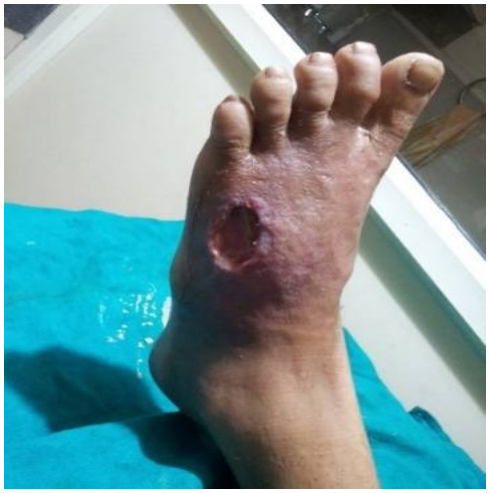
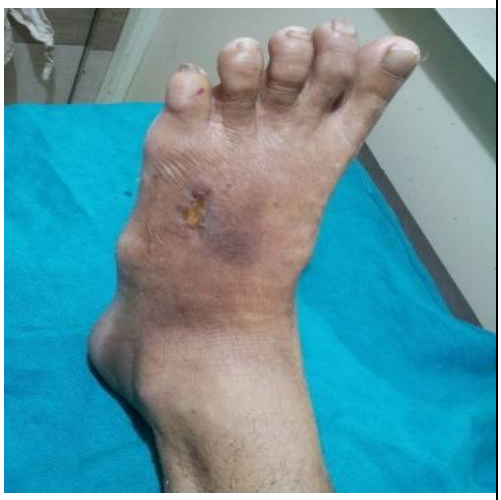


Figure 2 Pathogen species groups found in wound tissue

During first debridement, deep tissue obtained from the wound site was subjected to culture and antibiotic sensitivity. Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter lwoffi, Staphylococcus aureus, Staphylococcus simularis, Citrobacter freundii, Citrobacter sedlakii, were the common bacterial species isolated from wounds of the study subjects. More than 50 % of the wound was affected with up to 2 pathogen species.

	0 day	**60th day**
Honey group		
	Post debridement necrosectomy in a chronic non healing wound of a type 2 diabetic with neuropathy	**Contracted healing wound post honey dressings**

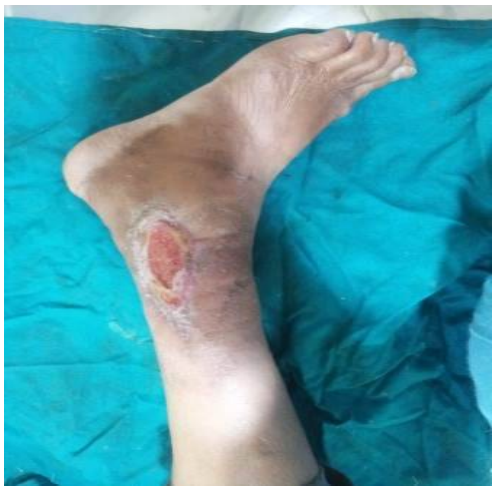
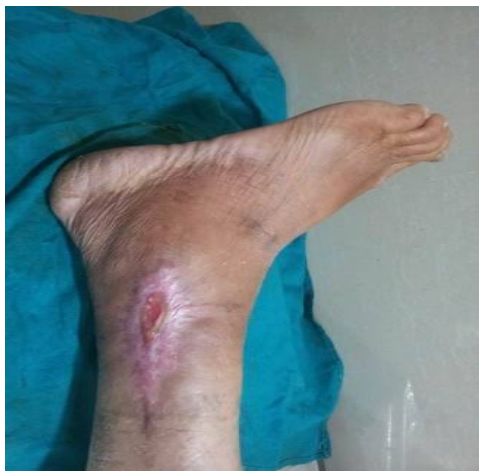
Saline group		
	Mildly infected chronic non healing ulcer post debridement in a neuropathic type 2 Diabetic wound	**Contracting healing wound post saline dressings**

Figure 3: Wound images before and at the end of treatment (60days)

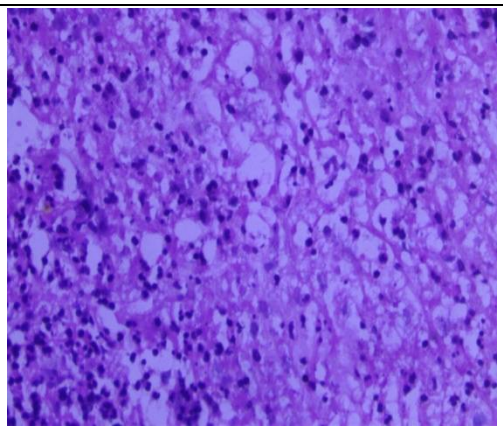
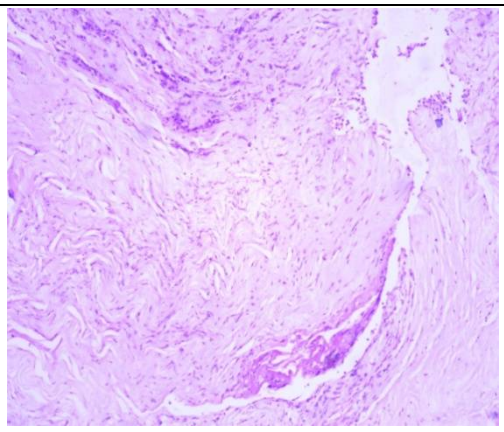
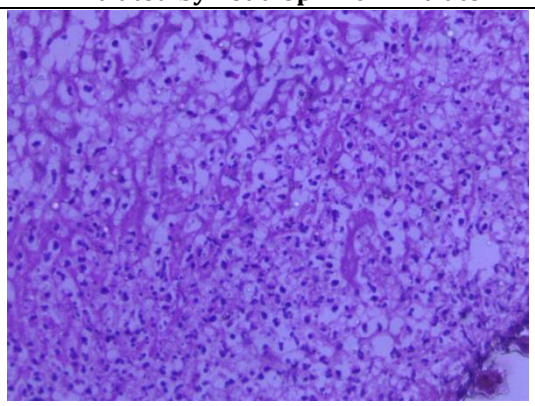
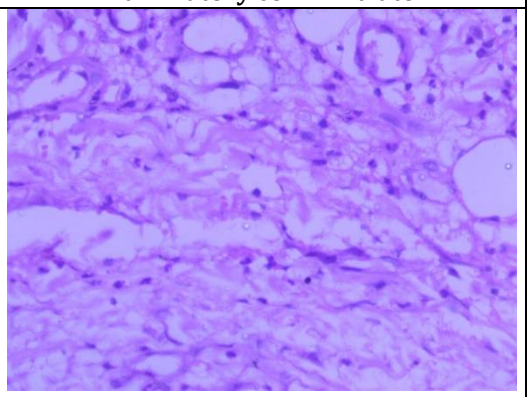
	0 day	**30th day**
Honey group		
	40X High poser showing edematous tissue infiltrated by neutrophilic infiltrate	**10X Low power showing fibrosis with mild inflammatory cell infiltrate**
Saline group		
	40X High power showing edematous tissue with polymorphonuclear inflammatory cell infiltrate	**40 X High power view showing Focal area of Fibrosis with Granulation Tissue**

Figure 4: Histopathological observations of the wound tissue.

Wound healing is measured as a contraction of the wound area on follow-up we found significant reduction in the wound area (by paired t test p = =<0.05), however on the 60th day the percentage contraction was observed more compared to other days, in the 90th day all most all the wound was cured. The mean percentage of contraction of the wound area was analyzed for the comparison of the efficacy of the two different treatments used for the wound dressing on 60th day. The mean wound percentage contraction of honey treatment group (88.1%) was significant than normal saline (75.2%) subjects (p =<0.05; Figure 4)

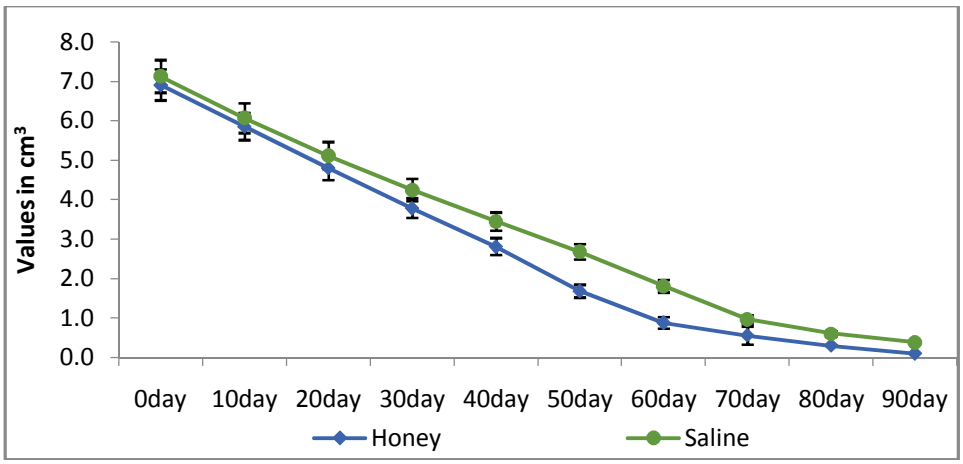


Fig 5: Wound area during the follow-up in different treatment groups.

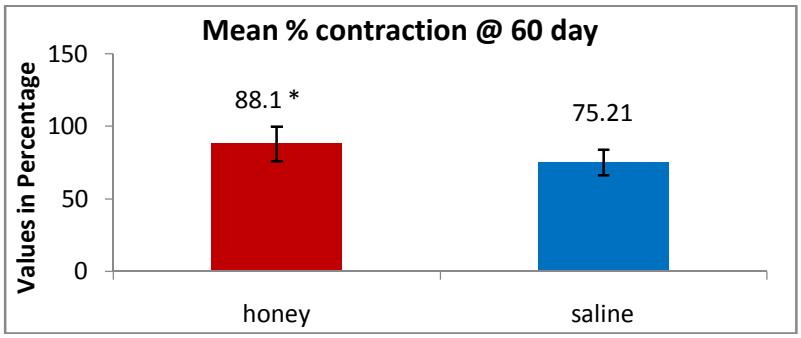


Figure 6: Comparison of percentage wound contraction on 60th day among various Treatment groups (p=value <0.05).

The time duration for ulcer healing was observed to be74.5 ± 14 days in honey group and 89.3±21.9 days in normal saline group. (p =<0.05).

DISCUSSION

Several studies documented use of honey for wound healing due to its manifold properties; moisturizing wound bed, antimicrobial, anti-inflammatory effect, hyperosmolarity which inhibits bacterial proliferation, facilitates debridement of necrotic tissues from wound, reduces cellular and wound oedema, promotes lymphocytic, macrophage activities, augments wound angiogenesis and granulation tissue, collagen growth and epithelialization of wound [13,14]**.** Honey is an effective wound antiseptic with broad spectrum antibacterial activity. It can clear infection through anti-inflammatory property, stimulating promoting immune system, stimulation of cell growth and antioxidant activity [15]**.** Compilation of earlier reports on honey as would healing agent documented topical application of honey shortens wound healing time [16].

The study subjects in our two treatment groups on admission exhibited no significant difference in respect to demographic profile, anthropometric parameters, clinical signs symptoms, liver function tests, renal function tests and lipid profile except for HBA1c, HDL cholesterol and SGOT values which were significant.

Participants mostly had type 2 diabetes with duration of diabetes being mainly in the range of 5- 10 years duration. Risk of diabetic foot complications is higher with increasing duration of diabetes type 2 patients which is more common than type 1 diabetes. Relatively more proportion of male participants in this study

may be because predominantly outdoor activities make them vulnerable to friction, trauma and repetitive forces of walking and related foot ulcers.

In our study the highest number of wounds were infected with Staphylococcus aureus (25; 10.4 %), and Klebsiella spp (15; 6.3%) with similarity in combination of pathogens too, Staphylococcus aureus and Klebsiella spp 40(16.7%), Acinetobacter spp, Citrobacter species, Klebsiella species & Staph species 35 (14.6%), Citrobacter species and Klebsiella spp 26 (10.8%) were seen.

In other studies, anti-bacterial efficiency of honey has been demonstrated against S. aureus, P. aeruginosa, MRSA, MSSA etc. in varying concentration [17-20].

Honey has broad spectrum activity against wound microbial flora [21,22]. The anti-bacterial and anti-fungal activities are attributed to high osmolarity, hydrogen peroxide producing property on contact with wound moisture, presence of defensin-1, 10-HDA, aromatic acids [23]. Honey reduces foul wound odor, oedema and quantity of devitalized tissue due to acidic pH, osmotic effect, reduces wound proteases, promotes autolysis by inactivating plasminogen inactivator allowing plasmin formation and digestion of fibrin [24].

The various symptoms of inflammation such as pain, itching and burning and signs of inflammation such as odor, discharge, redness and swelling in peri wound area were present on initial visit but disappeared after 3 to 5 days of debridement and applying honey dressing, suggestive of its strong anti-inflammatory and antioxidant effect on topical application.

In this study there was a rise in tissue antioxidants level as well during inflammatory and proliferative phase of wound which reduces ROS present locally. In diabetic patients, advanced end products of glycation and lipoperoxidation induce oxidative stress and inflammatory excess which is counteracted by antioxidant and anti-inflammatory properties of honey, due to presence of enzymes, phenolic acids and bioflavonoids [25,26]**.**

Wound healing was measured as contraction of wound area and gradual reduction in wound area was found. The mean wound area in subject of either treatment groups was not statistically significant. The highest ulcer size in studied groups was up to 10 cm^3.

On 60^{th} day of follow up, percentage contraction of wound was more in honey to compared to saline group and there was statistically significant difference ($p < 0.001$). Honey is known to stimulate clean good healthy granulation tissue with epithelialization by autolytic debridement, inhibiting bacterial growth, absorbing pus, destroying odor and promoting new growth due to it content of pollen, bee proteins and various enzymes.

In our study mean time duration (in days) to achieve complete healing in honey group was 74.5 days compared to 89.3 days for saline dressings. Honey removes interstitial fluid from edematous peri wound tissues by osmosis and improves blood supply to healing tissues.

Comparable results were reported in other studies. Compared to traditional wound dressings, the manuka honey impregnated dressings had shorter healing time in a randomized double-blind study on neuropathic DFU [27]. No major and minor amputation were done in both groups in our study, a finding in agreement with that reported in other studies. [27,28]. Another study with honey dressings on diabetic foot revealed that use of honey in diabetic foot ulcers had a significantly positive effect on healing response [29]. Significant reduction in healing time in diabetic foot ulcer patients was found in a large, randomized control trial on 375 patients in whom honey dressings were applied [30].

Significantly longer healing time was reported in the study done on neuropathic DFU with mild ischemia. It was found that honey had good effect on wound healing and found to reduce pain considerably along with reduction in dressing cost [31,32] All wounds in our study were neuropathic and none of wounds had ischemia (ABI,0.9)

CONCLUSION

In comparison to traditional normal saline dressings for DFU, natural compound honey dressings exhibited more efficacy for rapid healing of chronic wounds diabetic subjects. Use of honey for DFU healing is safe, cost effective and promises an improved quality of life without the need of amputation; a fact which is of importance in developing countries which have limited financial resources.

REFERENCES

1. T. G. Mariam *et al.*, (2017).“Prevalence of Diabetic Foot Ulcer and Associated Factors among Adult Diabetic Patients Who Attend the Diabetic Follow-Up Clinic at the University of Gondar Referral Hospital, Northwest Ethiopia, 2016: Institutional-Based Cross-Sectional Study,” *J Diabetes Res*, vol. 2017, pp. 1–82017, doi: 1155/2017/2879249.

2. F. Al-Maskari and M. El-Sadig, (2007). "Prevalence of risk factors for diabetic foot complications.," *BMC Fam Pract*, vol. 8, p. 59, doi: 10.1186/1471-2296-8-59.
3. Y. Maeda *et al.*, (2008). "Antibacterial activity of honey against community-associated methicillin-resistant Staphylococcus aureus (CA-MRSA)," *Complement Ther Clin Pract*, vol. 14, no. 2, pp. 77–82. doi: 10.1016/ j.ctcp.2007.11.004.
4. R. Mukherjee, S. Samanta, A. Jha, and P. G. Sarkar, (2018). "A Comparative Study of Effectiveness of Honey and Povidone Lodine in Healing of Surgical Wound," *International Journal of Contemporary Medicine, Surgery and Radiology*, vol. 3, no. 4, 200-209. doi: 10.21276/ijcmsr.2018.3.4.32.
5. K. R. Hixon *et al.*, (2019). "A Critical Review and Perspective of Honey in Tissue Engineering and Clinical Wound Healing," *Adv Wound Care (New Rochelle)*, vol. 8, no. 8, pp. 403–415. doi: 10.1089/wound.2018.0848.
6. R. Zubair and N. Aziz, (2015). "As Smooth as Honey—The Historical Use of Honey as Topical Medication," *JAMA Dermatol*, vol. 151, no. 10, p. 1102.doi: 0.1001/jamadermatol.2015.1764.
7. P. C. Molan, "Potential of Honey in the Treatment of Wounds and Burns," *Am J Clin Dermatol*, vol. 2, no. 1, pp. 13–19, 2001, doi: 10.2165/00128071-200102010-00003.
8. Y. C. Chan, S. Roy, S. Khanna, and C. K. Sen, "Downregulation of Endothelial MicroRNA-200b Supports Cutaneous Wound Angiogenesis By Desilencing GATA Binding Protein 2 and Vascular Endothelial Growth Factor Receptor 2," *Arterioscler Thromb Vasc Biol*, vol. 32, no. 6, pp. 1372–1382, Jun. 2012, doi: 10.1161/ATVBAHA.112.248583.
9. P. H. S. Kwakman, A. A. te Velde, L. Boer, D. Speijer, M. J. Christina Vandenbroucke-Grauls, and S. A. J. Zaat, "How honey kills bacteria," *The FASEB Journal*, vol. 24, no. 7, pp. 2576–2582, Jul. 2010, doi: 10.1096/fj.09-150789.
10. H. Aebi, "[13] Catalase in vitro," 1984, pp. 121–126. doi: 10.1016/S0076-6879(84)05016-3.
11. G. L. Ellman, "Tissue sulfhydryl groups," *Arch Biochem Biophys*, vol. 82, no. 1, pp. 70–77, May 1959, doi: 10.1016/0003-9861(59)90090-6.
12. C. C. Winterbourn, "Superoxide as an intracellular radical sink," *Free Radic Biol Med*, vol. 14, no. 1, pp. 85–90, Jan. 1993, doi: 10.1016/0891-5849(93)90512-S.
13. E. Delshad, M. Tavakkoli-Kakhki, and M. Motavasselian, "Successful Repair of Diabetic Foot Ulcer with Honey-Based Treatment: A Case Report," *Iran Red Crescent Med J*, vol. 19, no. 3, Dec. 2016, doi: 10.5812/ircmj.41939.
14. M. B. Hussain, "Role of Honey in Topical and Systemic Bacterial Infections," *The Journal of Alternative and Complementary Medicine*, vol. 24, no. 1, pp. 15–24, Jan. 2018, doi: 10.1089/acm.2017.0017.
15. D. Cianciosi *et al.*, "Phenolic Compounds in Honey and Their Associated Health Benefits: A Review," *Molecules*, vol. 23, no. 9, p. 2322, Sep. 2018, doi: 10.3390/molecules23092322.
16. F. E. Brölmann, D. T. Ubbink, E. A. Nelson, K. Munte, C. M. A. M. van der Horst, and H. Vermeulen, "Evidence-based decisions for local and systemic wound care," *British Journal of Surgery*, vol. 99, no. 9, pp. 1172–1183, Aug. 2012, doi: 10.1002/bjs.8810.
17. A. Mirzaei, K. S. Karamolah, M. P. Mahnaie, F. Mousavi, P. M. Moghadam, and H. Mahmoudi, "Antibacterial Activity of Honey against Methicillin-Resistant and Sensitive Staphylococcus Aureus Isolated from Patients with Diabetic Foot Ulcer," *Open Microbiol J*, vol. 14, no. 1, pp. 260–265, Nov. 2020, doi: 10.2174/1874434602014010260.
18. A. Moussa, D. Noureddine, H. S. Mohamed, M. Abdelmelek, and A. Saad, (2012)."Antibacterial activity of various honey types of Algeria against Staphylococcus aureus and Streptococcus pyogenes," *Asian Pac J Trop Med*, vol. 5, no. 10, pp. 773–776. doi: 10.1016/S1995-7645(12)60141-2.
19. I. N. Pasias, I. K. Kiriakou, A. Kaitatzis, A. E. Koutelidakis, and C. Proestos, (2018). "Effect of late harvest and floral origin on honey antibacterial properties and quality parameters," *Food Chem*, vol. 242, pp. 513–518. doi: 10.1016/j.foodchem.2017.09.083.
20. M. Ballal, I. Bairy, V. Shenoy, and P. Shivananda, (2012). "Honey as an antimicrobial agent against Pseudomonas aeruginosa isolated from infected wounds," *J Glob Infect Dis*, vol. 4, no. 2, p. 102.doi: 10.4103/0974-777X.96770.
21. J. M. Alvarez-Suarez, M. Gasparrini, T. Y. Forbes-Hernández, L. Mazzoni, and F. Giampieri, (2014)."The Composition and Biological Activity of Honey: A Focus on Manuka Honey.," *Foods*, vol. 3, no. 3, pp. 420–432.doi: 10.3390/foods3030420.
22. O. Sherlock *et al.*, (2010). "Comparison of the antimicrobial activity of Ulmo honey from Chile and Manuka honey against methicillin-resistant Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa.," *BMC Complement Altern Med*, vol. 10, no. 1, p. 47. doi: 10.1186/1472-6882-10-47.
23. Y.-J. Chu *et al.*, (2016)."Clinical outcomes of toe amputation in patients with type 2 diabetes in Tianjin, China," *Int Wound J*, vol. 13, no. 2, pp. 175–181. doi: 10.1111/iwj.12249.
24. R. W. Tarnuzzer and G. S. Schultz, (1996)."Biochemical analysis of acute and chronic wound environments," *Wound Repair and Regeneration*, vol. 4, no. 3, pp. 321–325. doi: 10.1046/j.1524-475X.1996.40307.
25. P. Molan and T. Rhodes, (2015)."Honey: A Biologic Wound Dressing.," *Wounds*, vol. 27, no. 6, pp. 141–51.
26. B. Minden-Birkenmaier and G. Bowlin, (2018). "Honey-Based Templates in Wound Healing and Tissue Engineering," *Bioengineering*, vol. 5, no. 2, p. 46. doi: 10.3390/bioengineering5020046.
27. A. V. Kamaratos, K. N. Tzirogiannis, S. A. Iraklianou, G. I. Panoutsopoulos, I. E. Kanellos, and A. I. Melidonis, (2014)."Manuka honey-impregnated dressings in the treatment of neuropathic diabetic foot ulcers," *Int Wound J*, vol. 11, no. 3, pp. 259–263. doi: 10.1111/j.1742-481X.2012.01082. x.
28. S. K. Hammouri, (2004). The Role Of Honey In The Management Of Diabetic Foot Ulcers, J.Royal Medical Service 11 [2] 10-15 .
29. A. R. Surahio, A. A. Khan, M. Farooq, and I. Fatima, (2009). "Role of honey in wound dressing in diabetic foot ulcer.," *J Ayub Med Coll Abbottabad*, vol. 26, no. 3, pp. 304–6.

30. M. Imran and M. Baig, (2015). "A Randomized, Controlled Clinical Trial of Honey-Impregnated Dressing for Treating Diabetic Foot Ulcer Antioxidant Characteristics and Oxidative Stability of Cow and Buffalo Milk based Dairy Products View Project Dietary habits, lifestyle pattern and obesity among young Saudi university students View project," . [Online]. Available: https://www.researchgate.net /publication /282253113
31. H. Mohamed *et al.*, (2015). "The efficacy and safety of natural honey on the healing of foot ulcers: a case series.," *Wounds*, vol. 27, no. 4, pp. 103–14.
32. P. C. Molan, (2006). "The Evidence Supporting the Use of Honey as a Wound Dressing," *Int J Low Extrem Wounds*, vol. 5, no. 1, pp. 40–54. doi: 10.1177/1534734605286014.